DENTURE STOMATITIS.

CLINICAL AND LABORATORY STUDIES.

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DECLARATION

I declare that this thesis is entirely my own composition.

The work described was carried out within a research team based in the Department of Oral Medicine and Oral Pathology at The University of Edinburgh. I was soley responsible for all clinical aspects of the investigations. A research assistant provided help with laboratory investigations. The lymphocyte transformation assays were performed by the HIV Immunology Laboratory at the Royal Infirmary of Edinburgh. Haematology and liver function tests were done by the Departments of Haematology and Clinical Chemistry respectively, at the Royal Infirmary of Edinburgh.

Vincent Bissell.

ABSTRACT

This thesis describes investigations conducted to study factors influencing the outcome of antifungal treatment of denture stomatitis. Clinical, mycologic, haematologic and prosthodontic factors were evaluated. The humoral and cellular immune responses were also studied. Two antifungal agents were compared: systemically administered fluconazole and topically administered amphotericin. A total of 29 patients were randomised to receive fluconazole 50mg daily for 14 days; 30 were randomised to receive amphotericin lozenges and cream for 28 days. Patients were assessed at the time of entry into the study and at one, four and twelve weeks thereafter. There were no significant differences in clinical response between the groups at any follow-up visit. The best clinical response was observed after four weeks whereas the best mycologic response was noted after one week. At the 12 week assessment clinical evidence of relapse and recurrence was a common finding irrespective of treatment. Overall the correlation between mycologic and clinical events was poor. Correlation was better in those patients who demonstrated very poor and very good clinical responses. Fluconazole appeared to be largely ineffective against *Candida glabrata*. Haematologic deficiencies were infrequent and appeared not to affect clinical outcome. Clinical response in

non-smokers was better than in smokers and this difference was significant at the one week visit. Denture quality although generally poor appeared to exert no marked influence over clinical outcome. Adverse reactions to treatment were uncommon in both groups. Little change was observed in serum and salivary total and anti-candidal antibodies throughout the study. However the humoral immune response in smokers appeared to be depressed in comparison with non-smokers. Candidal infection appeared to cause depression of the ability of the cellular immune response to respond to *Candida* antigen. This response would appear to be restored following antifungal therapy. The results of these investigations suggest that factors influencing the short and long term success of antifungal treatment of denture stomatitis are, the necessity for elimination of yeasts from the mouth by the therapeutic agent, effective denture hygiene, and smoking. Prosthodontic factors would appear to be less important.

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All who drink of this remedy recover in a short time, except those whom it does not help, who all die. Therefore it fails only in incurable cases.

Galen (2nd Cent.)

CHAPTER ONE

A REVIEW OF THE LITERATURE PERTAINING TO DENTURE STOMATITIS

1. Historical Aspects

The first accounts of denture stomatitis in the medical and dental literature date from around the turn of the present century. Prior to this the occurrence of the condition is speculative. A pre-requisite for the development of the disease is the presence in the mouth of a denture and it is known that the Phoenicians and Etruscans constructed partial dentures as early as the third and fourth centuries BC.[1] These usually consisted of human teeth fastened into the mouth with gold bands or ligatures. Candidal infections of the mouth were also known in ancient times. Hippocrates was almost certainly describing Thrush when referring to two cases of oral aphthae in individuals with debilitating illness.[2] Whether candidal infections occurred in association with primitive prostheses is unknown but it seems unlikely given that mucosa was not covered to any great extent.

Pierre Fauchard in "Le Chirurgien Dentiste", published in 1728, was the first to write extensively about prosthetic dentistry[1]. He described complete dentures carved from bone and retained by means of springs connecting the upper and lower denture. Carved bone and ivory were to remain the principal denture base materials for well over a century and, given the approximate nature of their fit and the force exerted by the springs, they must have resulted in trauma to the underlying mucosa. Porcelain and gold were also used as denture base materials at about this time but all artificial teeth remained a luxury only a few could afford.

Of more significance would be the advent of a denture base material which could be made to fit accurately and which could be produced cheaply. This material was to be vulcanised rubber, invented by Charles Goodyear in 1851. Vulcanite became the most widely used denture base material and with improvements in impression techniques and means of production, well fitting dentures, which could be retained without the aid of springs, became available to the masses.[1]

Amongst the first descriptions of what is recognisably denture stomatitis is that of McKenzie[3] who writes;

"If the patient wears a plate on the roof of the mouth and this be removed, the underlying mucous membrane will be found swollen and red"

he also considers that this is;

"a form of infection very common amongst those who use false teeth where the utmost cleanliness is not observed".

McKenzie was writing after the introduction of vulcanite and given the increased availability of dentures the condition must have been increasingly observed by dentists and physicians. It is understandable that Lain in 1932 considered the constituents of the material an underlying cause.[4]

Cahn[5] was probably the first to consider that the condition might be associated with yeast infection, a view shared later by Bartels[6] and Stones[7]. Lyon and Chick[8] demonstrated the efficacy of antifungal therapy, thus implicating *Candida* infection in the aetiology. In their experiment, cure and improvement were effected despite the fact that patients continued to wear their existing dentures and they concluded that neither the fit nor the material of the denture were directly responsible for the condition. They did however consider that denture trauma might be a predisposing factor.

In these early papers the beginings of a multi-factorial concept of the aetiology of denture stomatitis can be seen. Early treatment regimens consisted of leaving dentures out at night and soaking them in a mild

hypochlorite solution[5], in addition to the use of therapeutic agents such as Gentian Violet[5] and Sodium Caprylate Jelly[8].

2. Nomenclature

There are a number of terms which are synonymous with "Denture Stomatitis" in that they have been used in the dental literature as names for the same clinical entity. These terms are listed in Table 1. "Denture Sore Mouth" probably replaced "Rubber Sore Mouth" in general usage when vulcanised rubber ceased to be used as a denture base material. However Cawson[15] found that soreness was not a prominent feature of the disease and suggested that "Denture Sore Mouth" was a misnomer. Both Neill[16] and Cawson[17] proposed "Denture Stomatitis" as a more suitable name.

Both "Denture Stomatitis" and "Denture Induced Stomatitis" are satisfactory terms. "Denture Induced Stomatitis" is probably preferred by those who feel that "Denture Stomatitis" implies a condition of the denture rather than of the patient, although this does seem somewhat pedantic. Both terms lack precision in that they might be taken to refer to any inflammatory condition of the mouth related to the presence of a denture. Hence a lesion caused by

TABLE 1

DENTURE STOMATITIS: SYNONYMS

Rubber Sore Mouth[8]
Denture Sore Mouth[5]
Stomatitis Prothetica[9]
Stomatopathia Prothetica[9]
Denture Sore Mouth Syndrome[9]
Denture-induced Stomatitis[10]
Chronic Atrophic Candidiasis[11]
Denture-related Candidiasis[12]
Candida-associated Denture Stomatitis[13]
Chronic Erythematous Candidosis[14]

trauma from an over-extended denture flange could be included under this heading. However few, if any, authors on this subject have included such lesions in their descriptions of the condition.

There is a significant body of evidence to support the involvement of *Candida* organisms in the aetiology of denture stomatitis (vide infra). This has lead to the inclusion of terms such as "Candidosis" in names for the condition. However the terms suggest a precise diagnosis which cannot be made from clinical observation alone and must be proven by other means. "Chronic Atrophic Candidiasis" is not suitable since the mucosa is not always atrophic[18] and in any case a histological examination would be required to demonstrate this. "Chronic Erythematous Candidosis" is also problematic in that chronic erythematous lesions have been shown to affect non-denture bearing tissues.[19] The pathogenesis of such lesions is probably quite different to those of denture stomatitis and the use of the same name for both conditions may not be desirable.

There is further controversy over the use of "Candidiasis" or "Candidosis". The present consensus would appear to favour the suffix -osis which is consistent with the ending used for almost all other fungal disease names.[20,21]

To summarise, "Denture Stomatitis" is a simple, well understood name. It refers to a distinct clinical condition and is therefore as specific as it needs to be. In the context of an individual or a group with this clinical condition, in whom candidal infection is proven, a more precise term may be used and "Candida-associated Denture Stomatitis" seems to be as good as any.

3. Clinical Aspects

(a) Classification

Classifications of the condition have been based largely on the clinically observable inflammatory changes of the maxillary denture bearing mucosa. The clinical material of Nyquist[22] and Ostlund[23] fell into three categories: small localised areas of redness, more generalised redness covering the whole of the denture bearing area, being sharply demarcated from the surrounding, healthy, non-denture bearing mucosa, and a third form in which the mucosa had a granular appearance. Newton's[24] description is essentially similar and has given rise to Newton's types 1, 2 and 3, as a popular classification. This is a little surprising given that Newton's paper is aimed at

establishing a specific aetiology for denture stomatitis based on rather weak theoretical similarities with the histological and clinical characteristics of the Sweat Retention Syndrome. With this in mind he describes the localised form of inflammation as "pin-point hyperaemia" which surrounds the orifices of the ducts of minor salivary glands. Hence investigators may be guilty of using "Newton type 1" rather loosely when they wish to describe a patchy type of erythema. Budtz-Jorgensen and Bertram[25] chose the terms simple localised, simple diffuse and granular to describe the same three variants.

Bergendal and Isaacson[18] declined to include the localised form of erythema in their classification since they considered sufficient evidence exists to implicate trauma alone as the cause of this condition. The diffuse and granular type of inflammation they consider should be referred to as atrophic and hyperplastic respectively. This is despite the fact that in their investigations many cases of so called atrophic denture stomatitis showed normal epithelial thickness. They also admit that erythema may be the result of increased vascularity rather than mucosal atrophy. Such inconsistencies must lend support to the observation of Holmstrup and Axell[26] that classifications should be based on clinical terms.

(b) Symptoms

Patients with denture stomatitis may complain of soreness, a burning sensation, itching or tingling, all of which are usually localised to the inflamed area; also of dryness of the mouth and bad tastes.[15,18,22,25,27-31]

Nyquist[22] found such symptoms to be uncommon and Cawson[15] found the degree of soreness to be variable and unrelated to the severity of inflammation. However Ritchie et al[27] and Olsen[29] found 45% and 44% of their patients, respectively, had symptoms. Two studies have reported symptoms according to inflammatory type and both found soreness to be commonest in the diffuse type of inflammation (59%[25] and 36.8%[18]).

These results should be interpreted with caution as symptoms such as burning, dryness etc., also occur in individuals without denture stomatitis. Dorey et al[31] reported that 28% of their patients with denture stomatitis complained of subjective symptoms, as did 19.9% of the controls. Of these latter it was estimated that psychological factors were involved in about one third. On a practical level denture stomatitis, when present, will usually be considered as the most likely cause of such symptoms. There is some justification for this since studies have demonstrated that resolution of denture

stomatitis is often accompanied by resolution of symptoms.[15,29,32]

(c) Associated oral conditions

A number of oral conditions have been described in association with denture stomatitis, most commonly, angular cheilitis. Angular cheilitis has been reported in approximately one third to one half of patients with denture stomatitis (see Table 2). This is commoner than in individuals with healthy oral mucosa[18,35]. The relationship between these two conditions has lead some to the view that they are part of the same clinical disease entity[11] especially since *Candida* species have been shown to be involved in angular cheilitis.[36,37] However the infective aetiology of angular cheilitis is complex and *Staphylococcus aureus* would appear to be even more significant than *Candida*.[38,39] Despite this, treatment of denture stomatitis often leads to healing of the angular lesions also.[17,29,32]

Other oral candidoses have been associated with the presence of denture stomatitis, namely; thrush[15], glossitis[15,18,25,40] and candidal leukoplakia.[18,41] This does not seem surprising and may be a result of increased candidal loading in the mouths of denture

TABLE 2

INCIDENCE OF ANGULAR CHEILITIS IN PATIENTS WITH DENTURE STOMATITIS

Lyon and Chick 1957[8]	33%
Cawson 1963[15]	54%
Makila 1969[33]	37%
Ritchie et al 1969[27]	41%
Budtz-Joregensen and Bertram 1970[25]	
simple generalised	41%
granular	22%
Davenport 1970[34]	48%
Bergendal and Isaacson 1983[18]	
atrophic	31.6%
hyperplastic	34.5%
Wilkieson et al 1991[35]	54%

wearers, or of increased susceptibility to candidal infections in these individuals, or both.

4. Prevalence

Denture wearing, particularly complete denture wearing, is commoner amongst older individuals than it is amongst younger individuals. As a result almost all enquiries into the prevalence of denture stomatitis have been surveys of elderly populations. The population samples studied fall, with one or two exceptions, into three groups:

1. Randomised samples, chosen to be representative of the elderly population as a whole.[42-47]

2. Samples of those living in nursing homes, hospitals etc.,(institutionalised elderly).[35, 48-55]

3. Samples of those attending dental hospitals.[22,56]

The studies in group 1 are more commonly representative of the elderly living independently within the community rather than of the entire elderly population.

It might be useful to make comparisons between the results of studies in the different population groups

above. However even comparing studies within the same group presents difficulties. Examination techniques and diagnostic criteria have differed considerably, as have the methods by which the samples are chosen and the way in which the results are presented. The prevalence rates quoted may refer to the entire population studied or only the population at risk, i.e. denture wearers. These features, together with varying socioeconomic and demographic factors, may explain the considerable range of reported prevalence.

Of the type of study in group 1 the lowest prevalence reported was 16.3% in a rural community in the USA[47], whereas the highest was 65% in three Danish towns of differing size and location.[43] Hoad-Reddick, Grant and Griffiths[57] looked at a combination of institutionalised elderly and those living in the community, in a United Kingdom Health Authority area, where they found a prevelance of 7.3%. Tobias[58] surveyed residents in sheltered accommodation in West Essex where the prevalence was found to be 9%. In both of these UK studies the prevalence appears to be very low. An earlier UK study[44] found denture stomatitis in 20.5% of the elderly population and, from the information given, in approximately 29% of those wearing dentures. This figure is nearer the average from other countries.

The reported prevalence in the institutionalised elderly varies between 19%[53] (USA, Peru, Argentina and Israel) and 54%[54] (Scotland). Nyquist[22] found denture stomatitis in 27% of a series of dental hospital patients whilst Budtz-Jorgensen[56] reported 75% of women and 55% of men had denture stomatitis, also in a series of dental hospital patients. Dental hospital patients tend to be highly selected populations and differences can be expected.

Denture stomatitis has been reported as commoner in women[22,45,55,56] whilst other studies have found no difference in prevalence between males and females.[35,46,49,50] No studies have shown denture stomatitis to be commoner in men. It has been found to be increasingly common with age[52], decreasingly common with age[42,45,49,50] and to have a prevalence unrelated to the age of the individuals studied.[46,54,55] One reason given for the more often observed decreasing prevalence with increasing age is that older individuals may be less embarrassed about leaving their dentures out at night and using soaking agents for cleaning them. The prevalence of denture stomatitis has been shown to be higher in complete denture wearers than in partial denture wearers.[42,45,60]

A number of surveys have reported the proportions with

TABLE 3

PROPORTION OF INFLAMMATORY TYPES - FROM SURVEYS OF THE PREVALENCE OF DENTURE STOMATITIS (%)

Ref. No.	Localised	Diffuse	Granular
46	74	21	5
50	33	35.5	31.5
51	54.7	37.7	7.6
54	61.5	27.7	10.8
55	51.8	37.1	11.1
59	62.9	15.2	21.9

the localised, diffuse and granular forms of denture stomatitis and these are given in Table 3. The figures in each row total 100%, representing the total number of individuals with denture stomatitis, not the total population studied. The percentages were, in some cases, extrapolated from the numbers given. The majority of studies show the localised type to be the commonest followed by the diffuse type and then the granular type.

5. Aetiology

The aetiology of denture stomatitis has been said to be multifactorial[61,62]. The factors which are claimed to contribute to the aetiology of denture stomatitis will be reviewed under the following headings:

(a) Micro-organisms and denture plaque

(b) Trauma from dentures

(c) Allergy and primary irritant reactions.

(d) Host-related factors.

(a) Micro-organisms and denture plaque.

Writing in 1918 McKenzie[3] seemed aware that denture stomatitis was associated with poor denture hygiene. Cahn[5] was probably the first to consider the disease had a specific infective aetiology. Both he and Bartels[6] were able to consistently isolate *Candida albicans* from the mouths and dentures of patients with denture stomatitis. Later investigators were able to demonstrate that yeasts could be cultured far more frequently from the mouths of patients with denture stomatitis than from the mouths of denture wearers with healthy mucosa[8,15,25,49,54,63,64] and also that yeasts could be more often isolated from dentures than from mucosal surfaces.[35,49,64] However it is apparent from these reports that yeasts were cultured from a variable number of patients with healthy mucosa and there is a recognition that *Candida albicans* and other yeast species may exist in the mouth as harmless commensal organisms. Budtz-Jorgensen, Stenderup and Grabowski[43], in a random sample of the elderly Danish population, showed that yeasts could be isolated from the mouths of 93% of those with denture stomatitis. However the yeast carriage rate amongst denture wearers with healthy mouths was 86%. It is clear that a causative role for yeasts cannot be postulated simply on the basis of more frequent isolation in individuals with the disease.

Cawson[15] suggested that denture stomatitis was caused by an increase in the numbers of yeast organisms in the mouths of individuals with the disease. Davenport,[34] by direct microscopic examination of smears taken from the fitting surface of dentures showed that *Candida* occurred in significantly greater quantities in patients with denture stomatitis than in denture wearers with healthy mucosa. Further, very dense colonisation by *Candida* was observed on the denture fitting surface far more often than on the palatal mucosa, in both patients and controls. It was concluded that denture stomatitis is associated with proliferation of *Candida* primarily within plaque on the fitting surface of the denture. A number of other workers, using culture techniques and direct microscopy of smears, have provided evidence to support this view[25,65-72].

Arendorf and Walker[66] compared different techniques for detecting and quantitating yeasts in dentate individuals, denture wearers with healthy mucosa and those with denture stomatitis. A number of mucosal sites were sampled in addition to the denture fitting surface where relevant. The imprint culture technique gave the greatest yield of *Candida* at the various sites. The fitting surface of complete dentures, the tongue, palate and cheeks were the most frequently and densely colonised sites. There was a

highly significant increase in density of candidal colonisation of all mucosal sites in normal denture wearers compared with dentate subjects. In denture stomatitis highly significant increases in candidal densities occurred in both upper and lower denture fitting surfaces, anterior palate and posterior tongue. In the dentate the mean candidal colony count did not exceed 30 colonies per square cm of mucosa and in denture wearers the maximum was 49 colonies/cm^2. In denture stomatitis colony growth was frequently confluent. They conclude that a prosthesis encourages the presence and growth of candidal species which are strongly implicated in the pathogenesis of denture stomatitis. They suggest that the imprint culture technique might be used to differentiate between the carrier and disease states given the maximum colony counts described above. However the usefulness of this suggestion seems questionable and has been challenged[35].

Denture plaque would appear to be a rapidly forming microbial plaque consisting largely of bacteria[69,72]. Significantly greater numbers of bacteria have been demonstrated in the denture plaque of denture stomatitis patients compared with that of healthy denture wearing controls[69]. The plaque on the fitting surface has been shown to harbour significantly more yeasts than that on the buccal flange and it would appear therefore that

conditions beneath the denture favour yeast colonisation[68]. Although yeasts have been shown to constitute less than 1% of bacterial counts in denture plaque their numbers are many times greater in the plaque of denture stomatitis patients than in the plaque of denture wearers with healthy mucosa[69,72]. In addition it has been pointed out that yeast cells and hyphae in particular are considerably larger in size than most bacteria.

Using an impression culture technique a close topographical relationship between the location of candidal colonies and areas of palatal erythema has been demonstrated[25,66]. Santarpia et al[73] cast *C.albicans* specific growth media into dentures and were able to show this topographical relationship only in Newton type III cases. However there was a general correlation between the severity of inflammation and the number of *C.albicans* colonies on the agar replica. The density of candidal colonisation of the denture fitting surface has been shown to be closely correlated with the degree of palatal erythema[70,71]. The same studies showed a similar relationship between erythema and *Candida* colony counts in saliva. Tamamoto et al[70] also showed a correlation between denture plaque scores and the grade of denture stomatitis, and between fungal concentration and plaque accumulation. Tarbet[67] also reported a correlation

between plaque levels and erythema, and between *Candida* counts and erythema. However he was unable to show a correlation between denture plaque levels and *Candida* counts. He interprets this as indicating that plaque levels and *Candida* exert independent effects. However in this study the *Candida* were derived from the mucosa, not the denture surface, and this might just as easily account for the lack of correlation.

With regard to the various yeast species which have been isolated, no difference has been found between the yeast flora of patients with denture stomatitis and that of controls[43,64,69]. *Candida albicans* has been by far the most frequently isolated species in both health and disease[15,25,34,43,51,64-66,69,70,71]. In contrast one study found *Candida glabrata* to be the commonest species in the population sample studied[54]. Wilkieson et al[35] found that *Candida albicans* was the most frequently isolated organism in their study group as a whole but that in patients with denture stomatitis *Candida glabrata* predominated. The virulence of *Candida* species may vary and is thought to be dependent upon a number of factors[74]. Amongst the most important of these factors is adherence and *Candida albicans* has been shown to adhere more readily to both mucosal and acrylic surfaces than other species[75]. This may explain its apparent predominance in both carriers and diseased individuals.

Candida albicans would appear to be isolated in pure culture more often than in combination with other species[25,35,65] although it may be difficult to identify mixed growth unless specific culture media is used[76].

It has been shown that a number of different strains of *C.albicans* are involved in denture stomatitis.[77,78] These studies failed to produce conclusive evidence that a particular strain was involved in the aetiology. However Martin and Lamb[79] demonstrated that *C.albicans* isolates from the denture fitting surface and palate of patients with denture stomatitis were almost exclusively serotype A compared with a mixture of serotype A and serotype B isolates found on the buccal mucosa of the same patients and at all sites in controls. They concluded that infection is associated with the proliferation of a single serotype to the exclusion of the other and that this proliferation is localised to the site covered by the denture. There is also experimental evidence from animal and *in vitro* models that certain strains of *C.albicans* are more virulent than others.[80,81] McCourtie and Douglas[81] compared the adherence to acrylic and buccal epithelial cells of strains isolated from active infections and from asymptomatic carriers. Growth in media containing high concentrations of sucrose and galactose increased the adherence of infective strains many times, whilst only

small increases in carrier strain adherence were observed. The same infective strains were also more virulent in mice. It would appear that the infective strains were able to modify their surface composition in response to high concentrations of certain sugars in growth media and that this enhanced their virulence.

A number of studies have indicated that the pathogenesis of denture stomatitis is associated with the presence of *Candida albicans* in its hyphal form.[17,25,34] Evidence from an experimental model in monkeys would appear to support this view.[82] Alison and Douglas[83] examined the fitting surface of temporary soft lining materials, from patients with denture stomatitis, by light microscopy and demonstrated micro-colonies of *Candida* with hyphae projecting from the denture fitting surface.

On the other hand abundant hyphae have often been demonstrated in denture wearers with healthy mucosa[43,66,84] and blastospores have occasionally been found to be the only morphologic form present in patients with denture stomatitis.[66,84] By infecting mice with morphologic mutants of *Candida albicans*, Shepherd[85] has produced compelling evidence that either yeast or mycelial forms are capable of producing disease. Interestingly Holbrook et al[80] showed, in experimental oral infection of mice, that a virulent strain of

C.albicans produced few, short pseudohyphae whilst an attenuated strain produced abundant, long pseudohyphae. Rebora et al[86] found mycelial forms to be rare in experimental infection of human skin. Clearly the contention that hyphae are the sole pathogenic form of *C.albicans* is difficult to sustain.

It has been suggested that the morphologic form of *C.albicans* is merely a product of local environmental conditions, in particular the pH.[62,82] Denture plaque in patients with denture stomatitis is more acidic than that in healthy controls,[84,87] particularly following sucrose rinses.[84,88] However no relationship could be demonstrated between the pH of resting denture plaque and the occurence of hyphae.[84,88] The pH of denture plaque may well be associated with the pathogenesis of denture stomatitis but it does not seem to be responsible for the conversion of *C.albicans* from the yeast to the mycelial form in vivo.

Denture plaque has been studied by electron microscopy[83,89-93]. The descriptions differ in detail but essentially the plaque is largely bacterial and is attached to the acrylic surface via a pellicle; yeast organisms are rare. The pellicle has been further characterised and would appear to differ from that formed on enamel[94]. In patients with denture stomatitis the

pellicle was found to contain serum products, degradation products and *C.albicans* cell components that were not detected in the pellicle from healthy patients. Catalan et al[89] compared plaque from patients with denture stomatitis and from those with healthy mucosa. Patients with denture stomatitis had thicker plaque which contained yeasts. Theilade and Budtz-Jorgensen[90] found no substantive difference between the denture plaque of those with denture stomatitis and those with healthy mucosa. In one sample the plaque contained only yeasts whilst no yeasts were seen in any other. This was despite demonstrating the presence of yeasts, by light microscopic examination of smears, in the plaque of all patients with denture stomatitis.

Frank and Steuer[91] found that thick denture plaque tended to consist largely of bacteria with only scattered yeasts whilst in thin plaque yeasts predominated. In this study only the plaque of patients with denture stomatitis was observed. Cytological signs of degeneration have been observed in plaque yeasts when in close association with large numbers of bacteria, suggesting yeast-bacteria antagonism[91,92]. It would appear that the intercellular spaces of the palatal epithelium harbour bacteria and yeasts[92,93] and that renewal of denture plaque occurs by proliferation of organisms from this site[93]. Frank and Steuer[91] often observed micro-organisms present

between the yeast layer and the pellicle and suggested that bacteria may be important in the attachment of yeasts to various surfaces in the mouth. Bagg and Silverwood[95] found that some common oral bacteria will bind to *C.albicans* and may act as a bridge in the attachment of *Candida* to the oral mucosa.

It would appear from the findings of electron microscopy studies that denture plaque is extremely heterogenous, with bacteria predominating in some areas, yeasts in others. Bacteria may be important in allowing the initial attachment of yeasts to denture plaque but if local conditions favour bacterial growth, yeasts may suffer as a result, and vice versa.

Palatal candidosis has been induced by the inoculation of *Candida* beneath acrylic plates in monkeys[82,96-98] and rats[99-101]. In monkeys, an erythematous response was produced with a similar histologic appearance to denture stomatitis in man[82]. However this resolved spontaneously after 2-3 weeks and could only be sustained by repeated inoculation and use of topical tetracycline. In one animal wearing a plate but with no *Candida* inoculum beneath it, localised erythema occured around the orifices of the palatal minor salivary glands. Two studies using a rat model have divided the animals into four experimental groups[99,100]; untreated animals,

animals wearing a plate but with no *Candida* inoculum, plate plus inoculum and inoculum only. Olsen and Bondevik[99] reported the most severe inflammation in the plate plus inoculum group. Sporadic inflammation was seen in other experimental animals but *Candida* was isolated from many of the animals prior to the study which complicates interpretation of these results. In the study by Shakir et al[100] no yeasts were isolated from the rats prior to the start of the study. They found that inflammation occurred only in the animals wearing a plate with *Candida* inoculated beneath it. This began as localised inflammation and spread to involve the whole of the area covered by the plate. The inflammatory response resolved when the plates were removed but *Candida* persisted in the mouths of the rats[100,101]. When the plates were subsequently re-inserted, without a further inoculum, the inflammation returned[101]. This was interpreted as indicating that the plate caused conversion of the *Candida* to its more pathogenic form (hyphae). However no attempt was made to quantify the number of yeasts present at any stage and an increase in the number of yeasts, held in close approximation to the mucosa, seems just as likely an explanation for the sequence of events observed. Some studies demonstrated hyphal invasion of the mucosa[100,101] whilst others did not[82,99].

Relatively few studies have been unable to show a relationship between candidal growth and denture stomatitis[9,18,51]. Nater et al[9] used an unusual method of isolating *Candida*, in that culture media were cast in the form of the upper denture and then applied to the palatal mucosa. Bergendal and Isaacson[18] also used a somewhat indirect method. In the epidemiological study by Schou et al[51] the overall recovery of *Candida* was low at 38.6% of the population, which must cast doubt on the methods used. Monaco and Pickett[102] denied the involvment of *Candida* in the hyperplastic form of the disease, although this conclusion was based on the absence of hyphal invasion alone.

The role of bacteria has received scant attention despite the fact that bacteria are generally far more numerous in denture plaque than yeasts[69,72,90]. The bacterial flora would appear to consist of Gram-positive cocci and short rods both in patients with denture stomatitis and in controls[69,72]. However Koopmans et al[103] did find differences between the bacterial flora of patients and controls. In individuals with denture stomatitis the percentage of the bacterial flora which consisted of cocci was lower than in healthy, denture wearing controls. The denture stomatitis group had higher percentages of obligate anaerobic bacteria in both denture and palatal plaque. Invasion of the epithelium

and sub-epithelial tissues by bacteria has been demonstrated[104]. On the other hand, when smears from the palate and dentures of denture stomatitis patients were examined, leucocytes were seen more frequently in association with yeasts than in association with bacteria[43]. This suggests that yeasts rather than bacteria are responsible for eliciting the inflammatory response. Nonetheless bacteria are important in the formation of denture plaque[91,95]. The possibility that bacteria themselves may have a direct pathogenic effect in denture stomatitis cannot be ruled out and requires further study.

Whatever the specific nature of denture plaque, clinical[25] and epidemiological[49-51,55] studies have shown that plaque occurs in significantly greater quantities on the dentures of patients with denture stomatitis than on the dentures of individuals with healthy mucosa. Studies have also shown a significant positive correlation between plaque accumulation and palatal erythema scores[67,70]. Ambjornsen[46] showed an increased prevelance of denture stomatitis in those with poor denture hygiene but this was not statistically significant. However Mikkonen et al[105] and Nyquist[106] found that the frequency of denture brushing did not significantly effect the prevalence of denture stomatitis. It has been suggested that the poor denture

cleanliness seen in denture stomatitis patients may be due to leucocyte emigration and the continuous shedding of epithelial cells from inflamed mucosa rather than to neglected hygiene[25]. Schou et al[51] were able to find no relation between denture plaque and brushing habits, whereas they were able to demonstrate a significant relation between denture plaque, soaking habits and the presence of denture stomatitis. This seems to indicate that brushing is not the most efficient means of removing denture plaque, at least when frequency of brushing is used as a measure.

(b) Trauma from dentures.

The first serious investigation of the role of trauma from dentures in the aetiology of denture stomatitis was that of Nyquist[22]. He examined over one thousand patients and assessed the marginal fit, surface fit, centric occlusion, articulation and vertical dimension of their complete dentures. Fit was recorded as stable or unstable and the occlusion was either correct or traumatising. This demonstrates one of the difficulties of investigating the effect of denture trauma, namely the rather crude and subjective means of assessment. Nyquist seems to have gone to some trouble to calibrate his observations against those of other clinicians and

claimed a remarkably small degree of discrepancy. He demonstrated that as the number of traumatic denture factors increased, so did the incidence of denture stomatitis. However there was no correlation between the extent of the area of erythema and the degree of trauma. Neither was any attempt made to measure denture hygiene or candidal infection.

Bastiaan[107] carried out a very similar study to Nyquist and their results with respect to trauma were in agreement. However he also assessed denture hygiene and the presence of *Candida*. He found patients with denture stomatitis to have poorer hygiene and a higher incidence of *Candida* colonisation compared with controls. Both Bastiaan[107] and Budtz-Jorgensen and Bertram[25], in addition to assessing the dentures, also assessed the alveolar ridges with respect to their ability to offer good retention and stability for dentures. Budtz-Jorgensen and Bertram[25] showed that poor ridge morphology predisposed to denture stomatitis. The localised form of inflammation was apparently associated with unbalanced occlusion and low *Candida* colony counts. On the other hand colonies grown using an impression technique correlated closely with the localised areas of inflammation. This could indicate that trauma predisposes to yeast infection. Trauma may be due to micro-roughness rather than macroscopic defects.[108]

Newton[24] suggested that a closely fitting denture would restrict the flow of saliva from the ducts of the palatal minor salivary glands and this would give rise to the appearance of pin-point hyperaemia around the duct orifices. This does not appear to be the same clinical entity as the localised, patchy form of erythema described by others[22,25]. Newton goes on to suggest that the generalised form of inflammation is caused by lateral spread of the saliva into the tissues, which is less plausible. Ritchie et al[27] have disputed this theory claiming that denture stomatitis begins in the anterior palate where there are no salivary glands. Newton's paper was entirely theoretical and contained no original data. It serves to illustrate that differences can arise in the definition of "trauma". It would seem that a traumatic denture can be either closely or loosely fitting. If trauma is related to instability as has been suggested[22,107], the incidence of denture stomatitis ought to be much higher in the mandible where the residual ridge is frequently extensively resorbed. Nyquist[22], in his large series, found no cases of denture stomatitis in the mandibular mucosa. This would accord with clinical observation and the fact that very few investigators even mention the lower denture bearing area. Despite these contradictions a number of studies

have indicated a relationship between traumatic denture factors, such as poor fit and occlusion, and the incidence of denture stomatitis[22,25,42,46,63,107,109]. There is an alternative interpretation of these studies, which is that inflammation induced by factors other than trauma causes changes in tissue shape which itself leads to poor fit and occlusion of the dentures.

Not surprisingly the incidence of denture related lesions is increased in patients who visit their dentist infrequently[47,105]. This may be related to the age of the dentures[46,105] which become progressively more traumatic as the ridges resorb and occlusal surfaces wear, or it may be due to a lapse in denture hygiene in the absence of professional encouragement[105]. Of course both factors could be involved. Unfortunately patient satisfaction with dentures seems to increase the longer they are worn[110] and the need for regular denture servicing is not perceived by denture wearers[44,58].

Pin-point hyperaemia of the ducts of the palatal salivary glands was produced in one monkey by covering the palate with a closely fitting acrylic plate[82]. However the animal was not gnotobiotic and therefore the role of of infection in this case remains uncertain. In rats, inflammation could only be induced beneath acrylic palatal appliances when *Candida* was inoculated on the

fitting surface[100,101]. The inflammation was initially patchy and later became diffuse[100].

The prevalence of denture stomatitis has been shown to decrease with increasing age[42,45,49,50]. Nyquist[22] made the same observation and suggested that this might be due to decreasing muscle power in elderly individuals, who were thus less likely to traumatise the denture bearing mucosa as a result of occlusal overloading. It has certainly been shown that subjective soreness beneath dentures may be the result of stress-induced muscle activity[111] and psychological factors may also be involved[9]. The role of occlusal loading in the aetiology of denture stomatitis remains speculative but would appear to be worthy of further investigation.

Samaranayake et al[112] showed that the adhesion of *Candida* to acrylic strips was enhanced by coating the strips with serum and reduced by coating with mixed saliva. They suggested that denture trauma may result in the production of an inflammatory exudate which would enhance adhesion to, and therefore colonisation of, the denture fitting surface by *Candida*. Saliva would appear to have a protective effect, and this would help to explain the very low incidence of denture stomatitis beneath lower dentures[22] where, due to the inevitable

poor denture retention, ingress of saliva is possible. The barrier properties of oral mucosa are affected by denture wearing[113] and indeed permeability is considerably increased when the mucosa becomes inflamed[113,114]. This is another possible means of interaction between trauma and infection; traumatised mucosa being more permeable to soluble microbial toxins and antigenic components.

The appearance of the granular or "papillary" form of denture stomatitis has lead some to the view that negative pressure (suction) is involved in its aetiology[63,115]. Nyquist[22] found papillary hyperplasia to be associated with the presence of a palatal vacuum chamber and Sheppard et al[110] found the incidence of papillomatous lesions to be increased in subjects with maximal denture retention. Also the role of yeasts in this condition has been refuted[102], although only on the grounds of absent hyphal invasion.

A number of investigators have been unable to demonstrate any significant correlation between trauma from dentures and denture stomatitis[9,50,55], although the study by Nater et al[9] related more to symptoms of soreness than to denture stomatitis as such.

(c) Allergy and primary irritant reactions.

Lain[4] reported cases of oral mucosal lesions which were presumed to be due to overcoloured or undercured vulcanite dentures. Such appliances were often provided by "cut rate dentists". Galvanic reactions between dissimilar metals present in the mouth were also said to be responsible for such lesions.

Allergic reaction to an acrylic denture base has occasionally been cited as the cause of denture stomatitis[116,117]. Acrylic resin contains a number of constituents which are potential haptens in the initiation of an allergic contact stomatitis[118,119]. Turrell[119] considered that Bradford's report[120] of an adverse reaction to newly fitted dentures was a case of allergy to dibutyl phthalate, an acrylic plasticiser. There are also reports of allergic reactions to autopolymerising acrylic resin[121,122,123] in which the high level of residual methylmethacrylate monomer is said to be the responsible agent. Such reactions should be distinguished from primary chemical irritation. Giunta and Zablotsky[121] considered their case one of true allergy since the response developed 24 hours after exposure to the material. They consider that a primary irritant reaction would have occurred much sooner.

Nyquist[22] showed that it was possible to produce an allergic reaction in the oral mucosa. However he investigated 248 cases of denture stomatitis and was unable to find a single case of allergy. Turrell[119] pointed out that methods of testing for acrylic allergy, such as strapping the patient's denture to the forearm, were quite unreliable as any positive response would likely be due to trauma. He tested 50 patients with denture stomatitis using filings from the patient's own dentures. He found eight cases of supposed allergy and these were all patients who had worn their dentures for many years. He considered the allergenic properties of these dentures to be due to contaminants absorbed during years of use. Subsequent dentures constructed from acrylic were well tolerated. It seems possible that the contaminant was microbial plaque. Danilewicz-Stysiak[116] reported four cases of acrylic allergy from 40 cases of "denture sore mouth". However the descriptions of the clinical status, history and patch testing technique are far from clear.

Fernstrom and Oquist[117] reported a case of denture stomatitis apparently due to acrylic allergy. Filings from the fitting surface of the patient's denture and an unpolished acrylic blank both produced a severe reaction on patch testing, whereas polished acrylic did not. They

postulated that the responsible allergen in the acrylic occupied the surface layer and that this was removed during polishing. This explained why the inflammatory reaction was confined to the palatal mucosa, since the fitting surface of a denture is not polished. In a subsequent investigation[124] the allergen was shown to be acrylic monomer and a satisfactory denture was made for the patient using the same acrylic but an altered curing technique. Further studies of the same patient[125] involved the use of filings from an unpolished denture which had never been used. Filings from the fitting surface produced a positive response to patch testing whereas filings from the vestibular surface produced a negative response. This indicated that the curing process was responsible for the location of excess monomer in the fitting surface acrylic and that polishing was irrelevant. It must be considered possible that the reactions seen in this patient were of a primary chemical irritant nature. Other such cases have been described[126,127], the patients usually complaining of an intense burning sensation within a few hours of the dentures being inserted. It would appear that, if a denture has been incorrectly processed, high levels of residual monomer may remain within it for a considerable period of time afterwards[126].

Allergy to a cobalt-chromium denture base has been reported[128] and was apparently due to the nickel content. Given that nickel allergy is quite common, reports of contact sensitivity to cobalt-chromium denture base alloys, which may contain varying amounts of nickel, are extremely rare. It would appear that patients who are sensitive to nickel may also be sensitive to cobalt[129]. There are also reports of allergy to dental gold alloys[130,131].

High levels of acrylic monomer are capable of producing an adverse response in the oral mucosa. Whether this is a chemical irritation or true delayed-type hypersensitivity may be difficult to determine. However the clincal picture is clearly different to the more usual chronic denture stomatitis. Allergy to metallic denture bases would appear to be very rare but may be suspected as a cause of inflammation beneath cobalt-chromium dentures in patients who are sensitive to nickel.

(d) Host related factors.

A variety of host related factors can predispose to denture stomatitis and may exert their effects either locally or via a systemic route.

Local factors

Denture wearing habits

Dietary sugars

Xerostomia

Smoking

Systemic factors

Nutritional deficiency states

Diabetes

Antibiotics

Corticosteroids

The importance of these factors in the aetiology of denture stomatitis relates mainly to the role of *Candida*. A considerable number of factors have been identified which may predispose to oral candidal carriage and infection[20,132-135]. Only those relevant to denture stomatitis will be considered here.

Denture wearing habits.

A number of studies have indicated that denture stomatitis is a more frequent finding amongst those who wear their dentures during both day and night, compared to those who leave their dentures out at night[22,27,34,42,55]. Love et al[42] found this difference to be particularly significant when patients with severe inflammation were compared. Cases of more

moderate inflammation occurred in equal numbers in those who wore their dentures at night and in those who did not.

The relationship between denture stomatitis and the wearing of dentures both day and night may be due to the constant effects of either denture trauma or microbial plaque. Williamson[136] demonstrated that patients who wore their dentures at night had much higher early morning *Candida* counts in saliva compared with patients who left their dentures out at night.

Some studies have failed to show any relationship between denture stomatitis and denture wearing habits[25,35,137], including what is probably the only prospective study of this problem[137]. In this investigation patients were provided with new complete dentures and divided into two groups. One group was instructed to wear the dentures constantly whilst the other group were told to leave their dentures out at night. When the patients were re-examined one year later the incidence of denture stomatitis was the same in both groups. Goss and Chau[138] showed that even wiring in dentures for prolonged periods did not necessarily have a deleterious effect on the oral mucosa.

Dietary sugar.

There is evidence to suggest that a high intake of sugars in the diet is associated with oral candidosis[139,140]. Olsen and Birkeland[140] found that sucrose rinses could aggravate existing denture stomatitis and initiate denture stomatitis in denture wearers with previously healthy mucosa. This change in mucosal health was accompanied by an increase in the density of *Candida*. *In vitro* studies have suggested that glucose promotes the growth of *Candida* in saliva[141,142] and that this growth is associated with a rapid decline in pH, which may be associated with the pathogenesis of oral candidosis[142]. Sugar substrate would also appear to enhance the ability of *Candida* to adhere to both acrylic and buccal epithelial cells[81,112].

Xerostomia.

The protective role of saliva against candidal infection, both through its mechanical washing effect and antifungal properties, is widely recognised[135]. However there is no direct evidence that xerostomia leads to denture stomatitis. *Candida albicans* may be found in significantly greater numbers in the mouths of patients with Sjogren's syndrome compared to healthy individuals[143]. Suppressed salivary flow in monkeys caused aggravation of inflammation produced by inoculating *Candida albicans* beneath acrylic palatal

plates[98]. Unsterilised human saliva inhibited the growth of *Candida*[141] and pre-coating strips of acrylic with mixed human saliva reduced the adhesion of *Candida*[112]. All of these studies support the concept that saliva has a protective role.

Smoking.

Two epidemiological studies have indicated that tobacco smoking is associated with a significantly increased prevalence of denture stomatitis[47,54]. Arendorf et al[41] found that tobacco smoking and denture wearing were important contributory factors in the aetiology of candidal leukoplakia. Approximately one third of patients with this condition also had denture stomatitis. Smoking has been claimed on one hand to increase[144], and on the other, to have no effect on[145], the rate of oral candidal carriage.

Nutritional deficiency states.

Cawson[146] was the first to suggest an association between anaemia and oral candidosis. Jennings and MacDonald[147] found that patients with haematological abnormalities developed denture stomatitis in association with less dense candidal colonisation than patients with normal haematology values. Saliva from patients with iron-deficient anaemia supported the growth of *Candida* better than did saliva from controls[148]. Iron

deficiency would appear to exert its effects through defects in lymphocyte function[148,149] although Fletcher et al[148] found impaired lymphocyte function in iron-deficient patients both with and without mouth lesions. This would seem to indicate the involvement of some other factor in the production of clinical disease. Jenkins et al[150] were able to find no significant differences in iron, folic acid and vitamin B12 deficiency between patients with denture stomatitis and healthy controls. In a retrospective study[151] cases of iron and folate deficiency were found in association with denture stomatitis. However correction of these deficiencies had little or no effect on the long term recurrence of the denture stomatitis.

Diabetes.

Candidal carriage has been shown to be more prevelant amongst diabetic than amongst non-diabetic individuals[152,153]. Whilst Tapper-Jones et al[153] were able to show that candidal density was also increased in diabetic patients, Lamey et al[152] could find no such increase in candidal density but did demonstrate an increased incidence of candidal infection amongst diabetic patients. Peters et al[154] could find no increase in the prevalence of candidal carriage or in the density of growth of *Candida* in diabetic patients, when compared with controls.

Saliva from diabetics has been shown to support the growth of *Candida* and this was correlated with the salivary glucose level[141]. Whilst investigators have claimed that candidal carriage rate amongst diabetic patients is dependent upon glycaemic control[155], others have denied such a relationship[152,153,156,157]. It should be borne in mind when interpreting these studies that methods of isolating *Candida* and of estimating glycaemic control have varied considerably. Of more direct significance, Phelan and Levin[158] were able to demonstrate no significant increase in the prevalence of denture stomatitis in those individuals with either diabetes mellitus or elevated plasma glucose levels compared with individuals with normal glucose metabolism.

Antibiotics and corticosteroids.

There seems to be general agreement that the use of broad-spectrum antibiotics in particular and corticosteroids, to a lesser extent, may predispose to the development of oral candidosis[20,135]. Knight and Fletcher[141] produced convincing evidence that antibiotics promoted the growth of *Candida* by inhibiting bacteria and thus removing the competition for salivary glucose. The hyperglycaemic effect of corticosteroids was considered responsible for promoting candidal growth.

McKendrick et al[159] investigated patients with chronic bronchitis who were receiving either continuous tetracycline, intermittent tetracycline or a placebo. They could find no relationship between the administration of tetracycline and the prevalence or relative numbers of oral *Candida*. Furthermore McKendrick[160] could find no significant relationship between tetracycline administration and the occurrence of denture stomatitis in what appears to be the same group of patients.

There is experimental evidence from an animal model to suggest that corticosteroids may exacerbate denture stomatitis, possibly by suppressing cellular immunity[97]. Other than this there would appear to be no direct evidence linking the use of corticosteroids to denture stomatitis.

6. Histopathology.

A number of investigators have examined biopsy specimens from the palatal mucosa of patients with denture stomatitis and reported their findings[18,23,161-163]. Their observations have been quite consistent and the main features seen are as follows:

1. Complete absence of the keratin layer or parakeratosis.
2. Epithelial hyperplasia with deeply penetrating rete pegs, alternating with areas of epithelial atrophy.
3. Intracellular and intercellular oedema within the epithelium.
4. Infilatration of the epithelium by leucocytes.
5. Chronic inflammation of the lamina propria usually extending into the basal epithelial layers.
6. No evidence of hyphae or blastospores invading the epithelium.

The granular form of denture stomatitis displayed much the same histological appearance as the generalised simple type except for the occurrence of papillary projections in the former[18,161,162]. The papillary projections consisted of either markedly hyperplastic epithelium with a narrow core of connective tissue or hyperplastic connective tissue with a covering epithelium of almost normal thickness[18]. The crypts between the papillae harboured microbial plaque and debris. Budtz-Jorgensen[161] divided his material into lesions caused by trauma and those associated with *Candida*, based on the presence of hyphal structures in smears and the clinical response to antifungal and prosthetic treatment. He could find no qualitative differences in

the histopathologic appearances of the two types of lesion. However, pronounced intraepithelial leucocyte infiltration and the occurrence of leucocytes in smears were seen more frequently in *Candida* induced lesions. This investigation also showed that it was possible to reverse the histologic changes by appropriate treatment. However, in the granular form, subepithelial inflammation persisted and this could be explained by the difficulty of removing the contents of the deep epithelial crypts described above. Another noteworthy feature of this study was the significant correlation between intraepthelial leucocyte infiltration and the quantity of yeast colonies isolated.

Schiodt[164] described an unusual clinical and histological variant of denture stomatitis in which there were intramucosal fistulae. These fistulae formed a network running horizontally beneath the mucosal surface.

The inflammatory cell infiltrate in denture stomatitis has been shown to consist mainly of T lymhpocytes, plasma cells and macrophages[165]. This cellular infiltrate is very similar to that found in chronic marginal periodontitis and chronic apical periodontitis.

Ostlund[23] found that in epithelium affected by denture stomatitis there was a moderate increase in the number of

cell mitoses compared with normal mucosa. In contrast Van Mens et al[166] found that denture stomatitis patients had a significantly lower mitotic index in their palatal epithelium compared with the epithelium of healthy denture wearers. In experimentally induced palatal candidosis in rats[167], palatal epithelial thickness and the number of mitotic figures were initially reduced but, subsequently, both epithelial thickness and mitotic activity showed a sharp increase. It may be that the increase in cell mitoses, the consequent increase in epithelial thickness and the shedding of the superficial layers of the epithelium are a protective mechanism. The same increase in epithelial thickness has not been uniformly observed in humans, indeed it alternates with areas of extreme atrophy[18,23,161-163]. The results of experimentally induced palatal candidosis in rats require careful interpretation. In this experimental model hyphal invasion of the epithelium seems to be not uncommon[100,101], whereas in man it is remarkable by its absence.[161]

7. Immunology

The inflammatory cell infiltrate in denture stomatitis suggests that both cell mediated and humoral immune

responses are involved in the disease process[165]. In addition IgG, IgA, IgM and complement factor C3 have been demonstrated in palatal mucosa in the granular form of denture stomatitis[168]. Antigen-antibody complexes may form which then activate complement and this process may be responsible for the inflammation seen.

A number of studies have demonstrated, using various techniques, that levels of serum antibodies to *Candida* are increased in patients with denture stomatits compared with controls[11,56,169,170]. According to Lehner[170], whilst most patients with oral candidosis demonstrated a raised IgG level in particular, patients with chronic atrophic candidosis also showed a marked increase in IgA and IgM levels. Fouche et al[171] investigated the serum antibody response in eight patients undergoing treatment with nystatin for denture stomatitis. In three patients, serum antibodies to *Candida* fell following clinical cure, then rose again gradually, but did not reach pre-treatment levels. This was interpreted as indicating that denture stomatitis in these three patients had been caused by *Candida*. In other patients the serum antibody response was more equivocal and this was taken to indicate that the disease in these patients had a different aetiology. However other studies have been unable to demonstrate any clear relationship between anti-*Candida* antibody titres in serum and either the

degree of palatal erythema or the density of candidal colonisation[56,169]. Bergendal and Holmberg[169] found that there was no change in serum antibody titres related to the effect of antifungal treatment of oral lesions. It is possible to detect levels of antibodies to *Candida* in many individuals without oral infection[56,169] and this may indicate past infection, infection elsewhere in the body, or simply carrier status. Furthermore zero or low titres may occur in infected persons[56]. The role of serum antibodies in denture stomatitis remains far from clear but they would appear to have a limited role in the diagnosis of this condition.

Titres of anti-*Candida* antibodies in saliva appear to be significantly higher in patients with oral candidosis, including denture stomatitis, than in non-infected controls[11,172,173]. Oral carriers of *Candida* seem to have intermediate levels of salivary antibody[172]. A significant relationship has been shown between titres of antibody to *Candida* in saliva and the number of colony forming units of *C.albicans* in saliva[173]. Also the level of anti-*Candida* antibody in saliva has been shown to fall in response to antifungal treatment[173]. The most important antibody class in the mucosal immune system is IgA. IgA has two subclasses, IgA1 and IgA2. Jeganathan et al[173] demonstrated that in controls the predominant subclass was IgA2 whereas in patients with

clinical infection IgA1 became the predominant subclass. In a number of patients antifungal therapy reversed this subclass alteration. Since IgA1 is the predominant circulating IgA subclass the possiblilty must exist that the increased levels of IgA1 in the saliva of the patients was derived from serum, via the lesions, rather than being a true change in the composition of the secreted IgA in saliva. In any case the significance of the findings is unknown. At least some of the secreted IgA would appear to be able to inhibit the adherence of *Candida* to epithelial cells[172] and it has been suggested that, although salivary antibodies are unable to eliminate *Candida*, they may contribute to its confinement to the mucosa[174].

In contrast to some of the above studies Wray et al[175] found that whilst levels of serum and salivary anti-*Candida* IgG rose in infected patients, levels of IgA tended to fall when compared with carriers and non-carriers. This effect was more pronounced in HIV positive individuals. The infected patients included some with denture stomatitis. These observations are difficult to explain. IgA synthesis in mice appears to be dependent upon a seperate subset of regulatory T-cells in Peyer's patches[176]. These T-cells predominantly help IgA synthesis but T-cells may also exist which suppress IgG synthesis. Consequently an antigen could induce a brisk

IgA response at the same time as it supresses IgG or IgM. This would seem to be beneficial as far as mucosal immunity is concerned but quite why or how the reverse of this could take place is not clear.

It is known that cellular immunity is important in combating systemic fungal infections whereas humoral immunity may be less important[177]. In an experimental monkey model, palatal candidosis tended to resolve spontaneously after two to three weeks[82]. On re-inoculation with *Candida* beneath the acrylic plate, the inflammation recurred but was more pronounced and this was assumed to be a delayed-type hypersensitivity response. In the same experimental model the cellular immune response was studied directly by observing *in vitro* leucocyte migration[96,97]. The cellular immune response was seen to reach its peak concomitantly with the resolution of inflammation. However the agglutinating serum antibody response occurred only after this resolution had taken place. In monkeys treated with azathioprine the clinical appearance of the lesion was changed from one of atrophic candidosis to one of thrush like lesions. Histologically there was hyphal invasion of the superficial epithelial layers. This inflammation persisted until after the azathioprine had been discontinued. The cellular immune response was suppressed by the azathioprine and did not occur until the drug was

stopped. The humoral immune response in these animals was early and strong but failed to clear the infection[96]. A similar picture was observed in monkeys treated with corticosteroids, which also suppressed the cell mediated immune response[97]. Both studies were taken to indicate that cellular rather than systemic humoral immunity is of primary importance in denture stomatitis. The cellular immune response itself may be responsible for the typical erythematous appearance of denture stomatitis. The role of secretory antibodies was not studied in these experiments and it seems likely that they were completely excluded from the area of the lesion by the fastened in plates.

It has been suggested that, in some patients, chronic *Candida* infection may lead to suppression of the cellular immune response[178,179]. Only 28% of patients with granular denture stomatitis showed inhibition of leucocyte migration compared with 94% of those with localised inflammation[178]. In some patients, inhibition of migration appeared to be restored by antifungal therapy. There was an inverse relationship between cellular immune response and serum agglutinins in patients with superficial candidosis. However the restoration of cellular immunity was unlikey to be antibody mediated since the levels of serum antibody remained unchanged in response to treatment. Davenport

and Wilton[179] found that the incidence of delayed hypersensitivity to *C.albicans* was lower in patients with denture stomatitis (12.5%), than in controls (26%). They therefore denied that a cell mediated immune reaction was responsible for the clinical appearance in denture stomatitis but suggested that immune suppression could be involved. In contrast, the function of leucocytes taken from patients with denture stomatitis was shown to be quite normal[180,181]. Also when cellular immunity was deliberately depressed in monkeys, the clinical and histologic nature of the lesions changed completely.

The immunology of oral candidal infections is obviously multi-faceted and therfore complex. Denture stomatits is no exception and much of the evidence is conflicting. There would appear to be a need for further studies of all aspects of the immune response and their interaction in the pathogenesis of this condition.

8. Treatment

Given that many of the issues concerning the aetiology of denture stomatitis remain unresolved it is not surprising that the treatment regimens which have been advocated and tested are many and varied. They fall into the following categories:

(a) Antifungals.

(b) Denture plaque control.

(c) Disinfectants.

(d) Prosthodontic treatment.

(e) Surgery.

(a) Antifungals.

Polyenes.

A number of studies have investigated the efficacy of the topically applied polyenes nystatin[12,15,17,32,104,182-186], and amphotericin[12,27,29,69,173,187] in the treatment of denture stomatitis. Some of these studies demonstrated

that patients could be completely cured[12,17.27,32], whilst others showed that although the majority of patients were improved, only a small number were cured completely from a clinical viewpoint[15,69,182,185,186]. Nairn[12] compared nystatin and amphotericin directly and they appeared to be equally efficacious. It should be remembered that the means of delivery of the drug has varied considerably between studies and this would seem to be an obvious explanation for the differences in outcome. A common finding, however, was high rates of relapse and recurrence following cessation of treatment[12,29,32,69,183]. The reasons for this tendency to relapse have been discussed by several authors and include re-infection from exogenous sources as well as from endogenous sites[15,17,182]. It would appear to be difficult to eradicate yeasts completely, even from the mouth[12,187].

Clinical improvement has usually been accompanied by mycologic improvement, although following withdrawal of the drug the density of candidal colonisation quickly returns to pre-treatment levels[27,32,182,185,187]. Interestingly, one study found the clinical signs to lag behind mycologic improvement by at least one week[185]. Both nystatin and amphotericin would appear to produce the greatest improvement in the clinical condition in those patients with initially high yeast counts[32,69].

Indeed a significant correlation was demonstrated between the pre-treatment yield of yeast colonies by cultivation and the therapeutic effect of nystatin[32].

The denture has been cited as an important source of re-infection[32,187]. In the study by Olsen[187] a number of treatment combinations were tested. Sucking amphotericin lozenges with the dentures out caused a significant reduction in the number of *Candida* colonies derived from the palate but, not surprisingly, the candidal colonisation of the denture fitting surface was relatively unaffected. It was necessary to soak the dentures in chlorhexidine to bring about a reduction in yeasts at this site. Clearly antimicrobial therapy needs to be directed at both the oral mucosa and the denture base. Resistance of yeasts to antifungal agents could be an explanation for failure of therapy in some cases. Ritchie et al[27] claim to have demonstrated resistance of yeasts to both amphotericin and nystatin *in vitro*. However Martin et al[186] tested *C.albicans* isolates from 500 cases of chronic atrophic candidosis and found no resistance to nystatin. They concluded that poor patient compliance was a more likely explanation for failure of antifungal treatment. Other factors which might be associated with relapse and recurrence have remained largely uninvestigated. Bergendal[183] followed up patients one year after treatment with either a

combination of nystatin and new dentures or new dentures alone. Nystatin treatment conferred no long term benefits. Since all patients were provided with new dentures the effect of trauma is difficult to assess. Persistent erythema was significantly associated with plaque and yeasts on the denture fitting surface.

Antifungal treatment has been shown to improve or resolve subjective symptoms[15,29,32] and other oral conditions[17,29,32] associated with denture stomatitis. It has also been shown to lead to reversal of the histopathologic[161] and immunologic[173] changes seen in the condition. Nystatin will apparently inhibit the growth of some streptococci isolated from denture stomatitis lesions[104] which may clearly be of benefit.

Antifungal drugs have been delivered to the mouth in some novel vehicles. Amphotericin included in denture fixative was ineffective[188], whereas a soft lining material incorporating nystatin seemed to be efficacious in the treatment of denture stomatitis[184]. Such means of applying antifungal agents might be effective in overcoming poor patient compliance with more conventional modes of treatment.

Azoles.

Topical miconazole gel applied to the fitting surface of the maxillary denture was effective in reducing erythema in denture stomatitis and this was accompanied by decreased candidal colonisation of various oral sites, as judged by an imprint culture technique[189]. The drug is also effective against some bacteria making it useful for the treatment of angular cheilitis[189]. Miconazole has been incorporated into a sustained drug delivery device in the maxillary denture[190], and half the patients so treated were cured. The half who were not cured had high initial levels of candidal colonisation and predisposing systemic factors could also be identified in these patients.

A systemic antifungal agent would have the potential benefit of improved patient compliance with treatment and also clearance of yeasts from endogenous sites which might be a source of re-infection. Systemically administered ketoconazole has been reported to be effective in the treatment of denture stomatitis in one patient[191]. However the use of this drug may be associated with serious side effects[192]. A more promising drug in this area is the recently introduced bis-triazole, fluconazole. Fluconazole is very well absorbed via the oral route and is widely distributed throughout body fluids, including saliva, having an

elimination half life of 30 hours[193]. The activity of fluconazole and ketoconazole against *Candida albicans* has been compared[194]. Whilst fluconazole was 16-fold less active than ketoconazole when tested *in vitro* it was 20-fold more active than ketoconazole *in vivo*. This remarkable finding was assumed to be due to differences in distribution and metabolism between the two drugs. The efficacy of fluconazole has been demonstrated in superficial fungal infections such as vaginal candidosis[195] and in oropharyngeal candidosis in patients who are HIV positive[196,197]. Lamey et al[198] described the successful use of fluconazole to treat a single patient with chronic hyperplastic candidosis. As well as being an effective antifungal agent it would appear that fluconazole may also reduce the adherence of *Candida* species to human buccal epithelial cells[199].

Fluconazole has been shown to be efficacious in the treatment of denture stomatitis in one open, noncomparative study[200] and in one placebo-controlled trial[13]. In this latter study fluconazole was shown to be a well tolerated and safe drug. One daily 50mg capsule of fluconazole proved effective in reducing palatal erythema and decreasing yeast colonisation. Clinically, only 16% of patients were cured whilst 73% were considered improved. In only three of 19 patients were yeasts completely eradicated. Four weeks after

completion of treatment clinical and mycologic relapse had occurred in the majority of patients. Fluconazole treatment resulted in marked qualitative changes in the yeast flora although there was no obvious correlation between the mycologic and clinical events. The patients were given no instructions with respect to denture hygiene or nocturnal denture wearing. It would be interesting to see if, in the presence of good denture hygiene, recurrence rates differed after treatment with fluconazole compared with a topical antifungal agent. The influence of re-infection from endogenous sites might then be determined.

(b) Denture plaque control.

Walker et al[10] demonstrated that the brushing of dentures was effective in reducing inflammation in denture stomatitis and that the use of amphotericin and Steradent(R) conferred no additional benefit. The patients had received instruction in denture brushing techniques. Schou et al[51] found no relationship between the frequency of brushing and the amount of denture plaque. Others found no relationship between the frequency of denture brushing and the prevalence of denture stomatitis[105,106]. Clearly the important factor in denture brushing is not how often, but how well, it is

done. For patients who lack manual dexterity or motivation, chemical soaking agents may be more appropriate. There are many such agents on the market and the commonest consist essentially of alkaline hypochlorite or alkaline peroxide[201]. They would all appear to be effective to some degree[201,202] but there is relatively little information on which to base comparisons[201]. Part of the beneficial effect of denture soaking agents is that the dentures are left out for a period of time.

An assumption that the matrix of denture plaque is similar to that of dental plaque, consisting largely of mutan, dextran and salivary glycoprotein, has led to interest in the use of enzymes as denture cleansers[203-205]. Enzymes, which might disrupt the plaque matrix, would appear to be effective in removing plaque from dentures[203,205] and in reducing its formation, particularly when combined with denture brushing[204]. The effect of more efficient plaque removal, when compared with the commercial cleanser Steradent(R), was to produce an improvement in the clinical condition of the mucosa in patients with denture stomatitis[203]. Acids have also been used to clean dentures with some success[201,206].

Brushing of the denture bearing tissues would appear to be effective in reducing inflammation in denture stomatitis[42,207], especially if the dentures are also removed at night[42]. Chamberlain et al[207] found that both tissue and denture brushing could reduce palatal inflammation. However tissue brushing seemed to be accompanied by an increase in denture plaque scores whilst denture brushing failed to reduce denture plaque. No explanation for this rather curious result was offered and it is difficult to understand how denture brushing brought about an improvement in the clinical condition if not through a reduction in denture plaque.

Glazing of the denture fitting surface was effective in reducing plaque formation and reducing palatal erythema in patients with denture stomatitis[208]. Unfortunately the glazing system tested does not appear to be particularly durable. Stafford et al[209] showed that if dentures were left to dry for a period of eight hours candidal colonisation of the fitting surface was significantly reduced. The common practice of soaking the dentures in water actually caused an increase in colonisation. The dimensional changes in the dentures, caused by drying, were so small as to be clinically insignificant.

Finally, in a study to evaluate an educational programme designed to improve oral hygiene behaviour amongst institutionalised elderly, it was found that the programme had little effect on any of the variables studied and that only about half the residents remembered the programme two months after its termination[210]. The conclusion drawn was that only well and not confused elderly could benefit from such programmes and that confused elderly required regular professional support with oral hygiene.

(c) Disinfectants.

Chlorhexidine has been used as both a denture disinfectant and a topical medicament[29,187,211-214]. In both types of application it has been shown to significantly reduce clinical signs of inflammation in patients with denture stomatitis[29,212,213] and to reduce yeast colonisation of the palate and denture[187,212,213]. However when use of chlorhexidine was terminated recurrence of inflammation and increased yeast colonisation soon followed in almost all patients[29,187,212,213]. Prolonged use of a 0.2% chlorhexidine solution as an overnight denture soaking agent prevented both clinical and mycologic relapse[214]. After five months, dentures

which had been soaked in this solution had accumulated heavy staining. This staining could be removed using a 0.6% solution of alkaline hypochlorite. The potential consequences of longer term use of chlorhexidine in these circumstances remain unknown. Brushing of dentures with a 1% chlorhexidine gel was more effective in preventing plaque formation than a commercial alkaline peroxide soaking agent, but no more effective than brushing with a placebo gel[211]. Chlorhexidine has been tested in combination with amphotericin, when it would appear to be effective[29,187], but *in vitro* testing has indicated that combining nystatin and chlorhexidine reduces the anti-candidal effect of both agents, possibly as a result of salt formation[215]. One way in which chlorhexidine may exert a beneficial effect is by reducing the adhesion of *Candida* species to acrylic resin[216].

Listerine antiseptic used as a mouthrinse and denture soaking agent was found to be as effective as a denture soft reline[217] and nystatin[218] in reducing inflammation in patients with denture stomatitis. Listerine was also effective in reducing yeast colonisation[218] although plaque scores were not reduced[217,218]. In neither of these studies were patients assessed after cessation of treatment and relapse may well have occurred.

(d) Prosthodontic treatment.

Long term resolution of denture stomatitis has been demonstrated following occlusal equilibration of complete dentures[22]. Also the use of tissue conditioning materials[32,219,220] and hard laboratory relines[219] has been shown to reduce the severity of, and in some cases resolve, palatal inflammation in patients with denture stomatitis. Some studies of tissue conditioning materials have not included long term follow-up examinations, neither have they involved assessment of denture plaque or yeast colonisation. This is important since changing the nature of the denture fitting surface may radically alter the ability of *Candida* to colonise it. In the study by Budtz-Jorgensen and Bertram[32] the benefit of tissue conditioning was most pronounced in the group of patients without verifiable *Candida* infection prior to the start of treatment. The criterion used for diagnosis of *Candida* infection was the presence of hyphae in palatal smears. Patients who did have a verifiable infection prior to treatment commencing responded poorly to tissue conditioning. In neither group of patients was the number of yeast colonies isolated by cultivation significantly changed by treatment. Patients who had hyphae present in palatal smears before treatment continued to have hyphae present after treatment. Unfortunately both smears and samples for cultivation

were obtained from the palatal mucosa and not the denture fitting surface, which might have been more interesting. Also in this study, in a number of patients with generalised inflammation who were treated with an antifungal drug, localised areas of erythema persisted after treatment. These patients were judged to have traumatising dentures and the observations support the contention that candidal infection is superimposed on traumatic lesions.

Permanent soft relining materials, such as Molloplast(R), may be used in the management of chronic soreness beneath mandibular dentures in particular[221]. Such soft lining materials may be associated with increased yeast colonisation[83,222]. An increased incidence of denture stomatitis has been reported in association with soft linings[221]. In contrast Wright et al[223] found no increased incidence of inflammation of the mandibular denture bearing mucosa associated with soft-lined lower dentures.

(e) Surgery.

Patients with the granular form of denture stomatitis may have the degree of erythema reduced by antifungal therapy but the papillary hyperplasia remains unaffected by such treatment[32]. Furthermore biopsies of these lesions show persistence of sub-epithelial inflammation following antifungal treatment[161], probably due to the presence of microbial plaque and debris in the deep epithelial crypts[18]. These patients may require surgery to resect the hyperplastic tissue[224,225]. Surgical treatment would appear to result in acceptable long term reduction in erythema provided that denture plaque control is good[183].

Comment.

Denture stomatitis is a relatively minor condition. It is, however, common and may occasionally give rise to significant discomfort. The evidence suggests that overgrowth of yeasts on the denture fitting surface is strongly implicated in the aetiology, although many studies have involved only semi-quantitative estimation of yeast density. Increased yeast colonisation appears to be directly related to increased amounts of denture plaque and both are related to increased severity of mucosal inflammation. Bacteria are far more numerous in denture plaque than yeasts but their role has rarely been investigated. The possibility exists that the pathogenic effect of denture plaque is non-specific and not related to any one particular organism. There is certainly evidence to suggest that poor denture hygiene is associated with the condition and that improved hygiene may lead to its resolution. It is surprising that no one has conducted denture hygiene withdrawal studies similar to those which established the causal relationship between plaque and gingivitis[226].

In favour of the specific effects of yeasts are studies showing a significant relationship between increased numbers of *Candida* and more severe histologic signs of

inflammation. The same would not appear to be true of bacteria. Of course proof of association between *Candida* and denture stomatitis is not proof that *Candida* is the cause. Further supporting evidence for the role of *Candida* comes from the knowledge that denture stomatitis is improved by antifungal therapy and that a very similar condition can be induced in animals by inoculation of yeasts beneath palatal plates.

Whilst few, if any, studies could be described as conclusive, the body of evidence seems to favour *Candida* as a major causative factor. However *Candida* has been investigated far more frequently than any other single factor and this may give a very misleading impression.

Trauma from dentures is difficult to assess objectively. This may partly explain why the evidence in support of a contributory role for trauma in the aetiology of denture stomatitis presents certain problems. The patchy form of erythema appears to be associated with traumatic dentures and has been shown to resolve following prosthetic treatment alone. However the patches of erythema are topographically related to areas of candidal colonisation. In addition, when *Candida* was inoculated beneath palatal plates in rats, the induced inflammation was initially patchy and later diffuse. The inference

which may be drawn is that the patchy form of erythema may simply be a less severe form of candidal infection. It has been suggested that yeasts may colonise initially in the region of traumatic lesions. Adherence of *Candida* could be aided by the presence of inflammatory exudate and subsequently candidal toxins and antigenic components would more easily pass through the more permeable epithelial barrier. This is speculation.

The logical question which remains unanswered is why, if instability and occlusal overloading are important aetiological factors, is denture stomatitis so rare in the mandibular mucosa? A denture may be traumatic by being occlusive and preventing ingress of saliva. The protective effect of saliva is well recognised. Unfortunately there is very little direct evidence to support this view. There is evidence to suggest that the environment beneath the maxillary denture supports the growth of yeasts and of one *Candida albicans* serotype in particular.

A number of host related factors have been implicated in denture stomatitis but few would appear to be of major significance. The evidence relating to denture wearing habits is inconclusive. Few studies have looked at nocturnal denture wearing in combination with denture hygiene. It would seem logical to assume that constant

denture wearing in the presence of excellent denture hygiene would have little deleterious effect, whilst in combination with poor hygiene it could lead to more severe inflammation. There is evidence that severe inflammation does occur more frequently in those who wear their dentures both day and night. Increased intake of dietary sugars would appear to increase candidal colonisation and lead to more pronounced mucosal inflammation.

Chemical irritation and allergy may be responsible for inflammation beneath dentures but both are rare and the clincal presentation would appear to be distinctive.

With regard to treatment, simple plaque removal appears to be effective. The major problem with antifungal treatment is the recurrence of the condition which occurs after treatment ceases. It seems to be difficult to clear *Candida*, even from the oral cavity, with topical agents. Compliance with tedious and prolonged regimens may be one reason for this. Also topical agents would have minimal, if any, effect on candidal colonisation at other host sites which may be a source of re-inoculation for the mouth. Whilst the results of antifungal therapy have been disappointing, many of the agents tested have had limitations.

Although recurrence has often been commented upon it has rarely been investigated in any depth. For instance the effect of antifungal treatment on different *Candida* species has hardly been mentioned and similarly the role of prosthetic factors in relation to recurrence has received scant attention.

The immunology of denture stomatitis remains poorly understood, not least because much of the experimental evidence is contradictory. This may be partly explained by the fact that many different methods have been employed. Surprisingly the role of the secretory immune system in this disease has received little attention.

CHAPTER TWO

AIMS AND PATIENTS

AIMS

The aims of the research presented in this thesis were:

1. To determine the efficacy of a recently introduced systemic antifungal agent (fluconazole) in comparison with an existing topical agent (amphotericin), with respect to palatal erythema and subjective symptoms.
2. To examine the relative rates of relapse and recurrence following each treatment, using the same clinical variables.
3. To determine the safety of and tolerance to the drugs.
4. To examine the mycologic effects of each drug as determined by the isolation of yeasts from a number of oral sites, quantitation of yeast organisms present in the mouth and the differential effects on yeast species.
5. To determine the effects of a number of patient and prosthodontic variables on the outcome of antifungal treatment of denture stomatits.
6. To examine the effect of antifungal treatment on the humoral immune response.
7. To conduct a preliminary study of the cellular immune response in patients with denture stomatitis.

PATIENTS

Patients were selected from those attending the prosthetics department of Edinburgh Dental Hospital. Those patients who were over 16 years of age and who presented with a clinical diagnosis of denture stomatitis were admitted to the study after explanation and consent. Patients were excluded from the study on the basis of one or more of the following:

1. The presence of oral disease other than denture stomatitis or candidosis which might be associated with the wearing of dentures.

2. Female patients who were either a) pregnant or b) lactating or c) of child bearing age and not using reliable contraception.

3. Known impairment of renal or hepatic function.

4. Use of any other antifungal treatment during the previous 10 days.

5. Use of barbiturates, coumarin anticoagulants or oral hypoglycaemic agents.

6. Known sensitivity to polyenes or the azole group of antimycotics, including imidazoles and triazoles.

7. Intention by the patient to donate blood during and/or for three weeks after the study medication period.

8. A history of alcoholism, drug abuse, psychosis, antagonistic personality, poor motivation or other emotional or intellectual problems likely to invalidate informed consent, or limit the ability of the subject to comply with protocol requirements.

9. Participation by the patient in any other study involving investigational or marketed drugs during the previous month or intention of doing so during the study.

Fifty nine patients were recruited. Of these 16 were male with a mean age of 51 years (range, 22 to 77 years) and 43 were female with a mean age of 55 years (range, 26 to 81 years).

CHAPTER THREE

CLINICAL METHODS

1. Trial Design.

At the initial visit patients were assessed clinically and mycologically, and blood was obtained for heamatologic and immunologic investigations. All information was recorded on a printed form and these were kept in a seperate file for each patient. Patients were then assigned to receive either fluconazole or amphotericin according to a pre-determined randomization list. Fluconazole was taken as a 50mg capsule, once a day, for 14 days. Amphotericin was administered in the form of lozenges which were to be allowed to dissolve in the mouth with the dentures removed, plus amphotericin cream which was to be applied to the fitting surface of the upper denture. The lozenges were to be sucked four times a day and the cream applied before the denture was replaced in the mouth, after sucking the lozenge. The amphotericin regimen was to be continued for 28 days. All patients received instruction in denture hygiene, which was to include soaking in a hypochlorite solution plus brushing with a commercial denture paste at least once a day. Patients were advised to leave their dentures out at night.

Patients were reviewed at one week, four weeks, and twelve weeks after treatment commenced. The same clinician examined all patients at all visits and was unaware of the medication prescribed.

2. First Visit

The initial visit consisted of a detailed assessment as follows:

(a) Patient Basic Data and History.

(b) Dental Assessment.

(c) Clinical Assessment.

(d) Mycologic Sampling.

(e) Blood Samples

(a) Patient Basic Data and History

(i) Date of birth, sex, ethnic origin, height and weight.

(ii) Smoking classification:
smoker/non-smoker/ex-smoker, cigarettes/cigars per day, grams tobacco per week

(iii) Alcohol consumption.

(iv) History of denture stomatitis:
duration of present episode, previous episodes.

(v) Previous treatment for denture stomatitis:

drug, dose, date started, date stopped, efficacy.

(vi) Relevant concomitant medical conditions, duration.

(vii) Concomitant drug therapy, dose, duration.

(b) Dental Assessment

(i) Dentures:

Upper - complete/partial, date acquired.

Lower - complete/partial, date acquired.

(ii) Assessment of dentures:

The following features were assessed:

Retention

Stability

Extension

Occlusion

Hygiene

Occlusal Vertical Dimension

The occlusal vertical dimension was recorded as either correct, increased or decreased, whilst all other factors were recorded as good, fair or poor. Examination of the dentures was based on the method described in a standard text on prosthetic dentistry[227], with modifications. Assessment of

retention, stability, extension and hygiene was confined to the upper denture only. Assessment of occlusion and vertical dimension obviously required the examination of both dentures together.

Retention of complete dentures was tested by grasping the incisor teeth and attempting to pull the denture away from the tissues. A similar attempt was made to dislodge partial dentures using whichever artificial teeth were present.

Stability of complete dentures was tested by placing the index fingers on the occlusal surfaces of the upper premolar teeth and attempting to move the denture laterally, antero-posteriorly and rotationally. A similar attempt was made to move partial dentures using the saddles present. The tendency of partial dentures to rock when finger pressure was applied to the occlusal surfaces of individual saddles was also included in the assessment where appropriate.

It was relatively straightforward for a single examiner (the author) to grade retention and stability on a three point scale, namely, good, fair or poor.

Extension of the denture base was assessed in relation to the full denture bearing area. This was more

important in relation to complete dentures and obviously may have had an influence on retention and stability. However full extension is also important in relation to denture support. Where partial dentures were worn assessment of extension involved a judgement as to what degree of extension of the saddles, or major connector, was required in terms of design. Correct extension was recorded as good, moderate over or under-extension as fair, and gross over or under-extension as poor.

Occlusion of complete dentures was assessed in the retruded position and in excursive movements. It was graded as follows:

good - balanced occlusion and articulation,

fair - balanced occlusion but unbalanced articulation,

poor - unbalanced occlusion and articulation.

Where partial dentures were worn the assessment depended on whether or not an acceptable intercuspal position (ICP) was indicated by the patient's natural teeth. Where there was no acceptable ICP the assessment was similar to that described for complete dentures. Otherwise they were graded as follows:

good - conform to ICP, provide contacts with opposing artificial and/or natural teeth and do not interfere with excursions,

fair - conform to ICP, do not provide adequate contacts and interfere with excursions,

poor - do not conform to ICP, or provide adequate contacts, and interfere with excursive movements.

No description can include every possible situation which might be encountered. The general strategy was to grade the occlusion according to its potential to traumatise the denture bearing mucosa. Assessment of occlusion was obviously not applicable to edentulous patients who wore no lower denture.

Hygiene was assessed by examining the fitting surface of the upper denture for the presence of visible deposits of plaque. No deposits were graded as good, light to moderate deposits as fair, and gross deposits as poor.

Occlusal vertical dimension was assessed by measuring the freeway space with the patient seated upright. A Willis gauge was used for this purpose and multiple measurements obtained until consistent readings were achieved. A freeway space of 3-4mm was taken to indicate a correct occlusal vertical dimension, whilst a lesser or absent freeway space indicated an increased dimension, and a greater freeway space indicated a reduced dimension. It was occasionally neccessary to confirm the findings by observation of facial proportions and the closest speaking space. Assessment of vertical

dimension was not applicable to edentulous patients who wore no lower denture or to some partial denture wearers where the vertical dimension was wholly determined by the natural teeth.

(iii) The number and position of the teeth present were recorded.

(c) Clinical Assessment

(i) Denture stomatitis was classified as:

Localised erythematous - patchy areas of erythema, not including pin-point hyperaemia alone.
Diffuse erythematous - erythema covering the whole of the denture bearing area.
Hyperplastic/granular - either of the above types of erythema with superimposed papillary hyperplasia.

(ii) Subjective symptoms of pain, burning sensations, abnormal tastes and dry mouth were recorded as absent, mild, moderate or severe

(iii) The following oral candidoses were recorded as present or absent:

Psuedomembranous candidosis
Acute erythematous candidosis
Median rhomboid glossitis
Angular cheilitis (left)
Angular cheilitis (right)
Chronic hyperplastic candidosis
Chronic mucocutaneous candidosis

(d) Mycologic Sampling

Samples for the cultivation of yeasts were obtained by vigorously swabbing the:

Hard palate
Fitting surface of the upper denture
Dorsum of the tongue
Floor of the mouth
Skin of left angle of the mouth
Skin of right angle of the mouth

An oral rinse sample was obtained to allow quantitation of yeasts[228]. The patient was asked to rinse the mouth for one minute with 10ml of phosphate buffered saline and then to return the solution to the container provided.

(e) Blood Samples

Approximately 35ml of venous blood was obtained for haematology, blood chemistry and estimation of total and specific anti-candidal serum immunoglobulins. Details of these investigations may be found in the chapter on laboratory methods.

3. Review Visits - Week One and Week Four

(a) Concomitant Conditions and Treatment

Any changes in concomitant medical conditions or drug therapy were noted, including the timing and duration of these changes.

(b) Adverse Events

The patient was questioned as to the occurrence of any adverse events which might have been associated with the trial medication. The nature of any adverse event was recorded as follows:

Adverse event - specify
Severity - mild, moderate, severe

Date of onset

Duration

Attributable to study treatment - yes, no

Outcome - disappeared with continued treatment

tolerated

symptomatic treatment given

dose reduced

study treatment temporarily suspended

study treatment stopped

(c) Clinical Assessments

Denture stomatitis, if present, was classified in the same way as at the initial visit. A change from diffuse erythematous to localised erythematous would have been indicative of improvement. Where no such change was observed any marked reduction in erythema, in comparison with the baseline visit, was recorded.

The patient was again questioned as to the subjective symptoms of pain, burning sensations, abnormal tastes and dry mouth, which were recorded as absent, mild, moderate or severe. The same variants of oral candidosis as on the first visit, were recorded as present or absent.

(d) Mycologic and Blood Samples

The samples taken for mycologic investigation at the baseline visit were repeated at week one and week four, as were the blood samples for haematology, blood chemistry and immunology.

4. Final Review - Week Twelve

This visit was approximately eight weeks after the completion of amphotericin therapy and ten weeks after the completion of fluconazole therapy. Review visits were designed to be equi-distant from the start of treatment rather than from its completion.

Changes in concomitant medical conditions and therapy were again noted as was the persistence of any adverse effects. Clincal and mycologic assessments were repeated and blood was obtained for immunologic investigation. Haematology and blood chemistry were not repeated at this visit (see Laboratory Methods).

5. StatisticaL Methods

Parametric data were analysed using the t test for sample means and the chi-square test for proportions. It should be noted that in the chi-square tests expected frequencies were occasionally low. Non-parametric data were compared using the Mann-Whitney and Wilcoxon rank sum tests.

Comment on Clinical Methods

Assessment of denture quality by grading a number of factors as good, fair or poor may be considered subjective and crude. Other studies have involved similar means of denture assessment and used similar gradations[22,46,109,110,229,230]. The three point scale of good, fair and poor seemed appropriate since this is the type of evaluation frequently used in clinical practice. Further gradations would imply a false level of precision. Similar methods of assessing denture hygiene have also been used in other studies[46,51,231]. The problems associated with subjectivity can be limited in two ways. Firstly, one operator can be responsible for assessing all patients. The judgments remain subjective but they should be consistent and, if the operator is unaware of the treatment prescribed, there is no reason to assume bias. Secondly, more than one operator can

assess each patient and the results compared. The problem with this method is how to arrive at an average between, for instance, "good" and "fair"? The first method was the one chosen for this study, with the author being responsible for the assessment of all patients.

In the diagnosis and classification of denture stomatitis, pin-point hyperaemia which might be attributable to traumatic occlusion of salivary gland duct orifices, was deliberately excluded if it presented as the only clinical sign.

With the oral rinse sample there was a dilemma as to whether the patient should wear the dentures whilst rinsing. There are no data available to suggest how this variable affects the resultant colony count. Rinsing was generally performed with the dentures removed but some patients found themselves unable to expectorate without their dentures in place. These patients were allowed to rinse whilst wearing their dentures. Comparison of colony counts between patients is therefore not possible. However, the important consideration is the change in the quantitiy of oral yeast in each individual patient during the course of the study. This is determinable from the data since patients were consistent in either wearing or not wearing their dentures whilst rinsing, throughout the study.

Denture hygiene was assessed at the initial visit only. This caused difficulties, in that it was not possible to assess to what degree hygiene improved over the course of the study, or the way in which this influenced clinical outcome (*vide infra*).

Assessment of palatal erythema again presents problems of subjectivity and consistency over the study period. At review visits, absence of erythema would clearly represent improvement, indeed the patient could be said to be cured. Reduction in the erythematous area, from generalised diffuse to patchy, could also be regarded as an improvement. Difficulties arise where the erythematous area remains the same but the degree of erythema changes. In the original protocol this problem was not anticipated but it soon became apparent that significant changes in the degree of erythema, from one visit to the next, would need to be recorded.

Previous studies have rated palatal erythema using scales such as: absent, slight, moderate or severe[13,29,182,185,186,217]. However the assessment remains subjective. The following scale was used by De Paola et al[217]:

0 **Normal** - Tissue has pink colour with stippling and texture that are considered healthy.

1 **Mild** - Slight erythema, no swelling or oedema, normal size and shape.

2 **Moderate** - Erythema with some oedema, loss of stippling and slight change from normal shape.

3 **Severe** - Acutely inflamed, erythema, oedema, definite changes in size, shape and consistency, hyperplastic projections.

The number of factors in each grade makes for difficulty in interpretation. For instance in grade 3 (severe), what is to be made of "acutely inflamed", and are hyperplastic projections absolutely neccessary for an award of grade 3? There is still the problem of ensuring that judgments remain consistent throughout the study. This has been addressed by photographing the palate at each visit and observing the prints in sequence at the end of the study[12,13,29,212]. This requires carefully standardised techniques for exposure and processing of the film. An error in either could render the results impossible to interpret. Again the problem of subjectivity remains.

Given the difficulties described it seems defensible to suggest that an experienced clinician could assess the degree of erythema in the same way as he would in a clinical situation. An assessment of absent, marked improvement or unchanged, would then be appropriate. This

was the method adopted in the present study. Although 59 patients were involved they were seen over a period of more than a year. The fact that at any one time a relatively small number of patients were taking part in the trial, made this form of assessment possible. With regard to symptoms, self grading by the patient, using a scale of absent, mild, moderate or severe, is acceptable clinical methodology[232].

Some clinicians may be of the view that diabetes can influence the clinical presentation of oral candidosis. In this study diabetic patients were not necessarily excluded unless they were taking oral hypoglycaemics, which may have interacted with fluconazole. Blood sugar levels were not estimated and the possible influence of defective glucose metabolism cannot, therefore, be determined from this study. This issue has been specifically addressed in the past, although with no consensus of opinion (see Chapter One).

CHAPTER FOUR

LABORATORY METHODS

1. Mycologic Samples

Swabs were transported immediately to the laboratory where they were placed in Sabouraud's broth and incubated for 48 hours, prior to plating out on Sabouraud's dextrose agar. After a further 48 hours the yeasts were speciated using a commercially available analytical profile index (API) system (La Balme les Grottes, 38390 Montalieu Vercieu, France), which permits speciation of yeasts on the basis of their ability to utilise specific substrate sources for growth[233,234]. Details of the method may be found in Appendix 3.

The oral rinse samples were centrifuged at 1700 x g for 10 minutes and the deposit resuspended in 1ml of sterile phosphate buffered saline. An aliquot of the concentrated rinse sample was inoculated onto and spread over a Sabouraud's dextrose agar plate which was incubated for 48 hours. The number of yeast colonies were then counted and the results expressed as colony forming units/ml of rinse.

2. Haematology

The purpose of the haematologic investigations was to establish the presence of any deficiency which might be contributing to the clinical condition and monitor any changes over the treatment period which might be attributable to the trial medication. The following estimations were carried out in the Haematology Department, Royal Infirmary of Edinburgh:

	Normal Range	
	Male	Female
Haemoglobin g/dl	13.00 - 18.00	11.50 - 16.50
Haematocrit ratio	0.40 - 0.54	0.37 - 0.47
Red Blood Cells $x10^{12}/l$	4.50 - 6.50	3.80 - 5.80
Platelets $x10^{9}/l$	150 - 350	150 - 350
White Blood Cells $x10^{9}/l$	4.00 - 11.00	4.00 - 11.00
Neutrophils %	40 - 75	
Lymohocytes %	20 - 45	
Monocytes %	2 - 10	
Eosinophils %	1 - 6	
Basophils %	Less than 1	
Serum B12 ng/l	170 - 1600	
Serum Folate ug/l	2.2 - 18.0	
ESR mm/hr	1 - 10	3 - 15

Serum vitamin B12 and serum folate were estimated at the first visit only.

3. Liver Function Tests

Because of the potential for certain azole drugs to induce hepatotoxicity, liver function tests were performed on all patients during the period of active medication. The following tests were performed in the Department of Clinical Chemistry, Royal Infirmary of Edinburgh:

		Normal Range	
		Male	Female
Bilirubin	umol/l	2 - 17	
Alanine Aminotransferase	units/l	10 - 40	
Alkaline Phosphatase	Units/l	40 - 100	
Gamma Glutamyl Transferase	Units/l	10-55	5-35
Total Protein	g/l	60 - 80	
Albumin	g/l	36 - 47	
Aspartate Aminotransferase	Units/l	10 - 35	

Estimation of serum ferritin was also performed in the Department of Clinical Chemistry (normal range, male: 16 - 350 ug/l, female: 8 - 300 ug/l). This was again related to the possibility of a deficiency state contributing to the clinical condition and was performed only at the initial visit.

4. Immunology

Total and specific anti-candidal antibody levels were measured in serum and saliva using the techniques described below. Serum was derived from 10ml of venous blood obtained from each patient at each visit. Approximately 5ml of whole saliva was also collected by expectoration, salivary flow being unstimulated.

(a) Radial Immunodiffusion

Total isotype specific serum and salivary immunoglobulin levels were estimated in duplicate, using a commercially available single radial immunodiffusion system (Behring, Marburg, Germany). This technique involves the placing of test samples into wells in plates of agarose gel containing antiserum to human IgG, IgA or IgM. Antibody in the test sample diffuses outwards into the gel until the point of antibody - antigen equivalence is reached, when a precipitin ring is formed. Using standard sera a standard curve can be constructed in terms of the diameter of the precipitin ring against the known immunoglobulin concentration. The immunoglobulin concentration of the test samples can then be determined by reference to this curve[235,236]. Standard reference sera of known IgG, IgM and IgA concentration was donated by the Blood Transfusion Service, Edinburgh.

(b) Enzyme-Linked Immunosorbent Assay (ELISA)

Class specific anti-candidal antibody levels were measured on two seperate occasions, in triplicate, using an ELISA developed in the Oral Pathology laboratory at the University of Edinburgh[175]. A description of the reagents used may be found in Appendix 1.

Polystyrene flat bottom 96 well microtitre plates (Flow Laboratories, Rickmansworth UK) were sensitised overnight at four degrees centigrade with whole *Candida albicans* in bicarbonate buffer at pH 9.6. The plates were then coated with test samples of serum or saliva in a volume of 100ul for 90 minutes. These test samles were diluted in PBS-tween containing 1% foetal calf serum (Flow Laboratories, Rickmansworth, UK). Serum dilutions of 1:8100 were used for IgG and IgM estimation and 1:2700 for IgA estimation. Saliva dilutions of 1:3 were used for estimation of IgG and IgM, and 1:9 for IgA estimation. A reference serum consisting of pooled sera from 10 patients and 10 controls was used in each plate in serial dilutions from 1:1000 to 1:24,300 for serum immunoglobulin estimation, and from 1:33,000 to 1:8,100,000 for salivary immunoglobulin estimation.

The plates were then coated with 100ul of goat anti-human alkaline phosphatase conjugated IgG, IgA or IgM (Sigma, Poole, Dorset, UK) diluted to 1:1000 in PBS-tween containing 1% foetal calf serum for 90 minutes. Plates were washed three times between stages with PBS-tween and tapped dry. Finally the wells were filled with p-nitrophenyl phosphate (Sigma, Poole, Dorset, UK) 1mg/ml in diethanolamine buffer. The rate of substrate degradation was indicated by the colour change which is proportional to the antibody concentration in the samples. The absorbance of the yellow colour produced by the reaction of the enzyme with p-nitrophenyl phosphate was read at 405 nanometres in a Titerek Multiscanner (Flow Laboratories, Rickmansworth, UK) after one hour. The optical density was assessed using commercially available computer software (Flow Laboratories, Rickmansworth, UK) and correlated with the reference sera which were arbitrarily assigned to contain 100 ELISA units of IgG, IgA and IgM. Serum and salivary anti-candidal antibodies were then expressed in ELISA units.

CHAPTER FIVE

RESULTS

The results will be presented under the following headings:

1. Patients.
2. Dental Assessment.
3. Denture Stomatitis.
4. Baseline Symptoms.
5. Concomitant Oral Cadidoses.
6. Efficacy Analyses - Patient Evaluability.
7. Clinical Efficacy of Fluconazole and Amphotericin.
8. Mycologic Efficacy of Fluconazole and Amphotericin.
9. Correlation of Clinical and Mycologic Response.
10. Clinical Outcome in each Classification of Denture Stomatitis.
11. Clinical Efficacy. The Effect of Sex and Smoking.
12. Denture Wearing Habits.
13. Clinical Efficacy. The Effect of Prosthetic Factors.
14. Haematology and Liver Function Tests.
15. Yeast Species.
16. Humoral Immune Response.
17. Adverse Reactions to Treatment.

1. Patients

Of the 59 patients entered into the study, 29 (8 males, 21 females) were randomly selected to receive fluconazole and 30 (8 males, 22 females) to receive amphotericin. The average age of fluconazole patients was 50 years and that of amphotericin patients was 60 years. About half the fluconazole patients and one third of amphotericin patients were smokers. Alcohol consumption was apparently low in both groups. Demographic details are summarised in Tables 4 and 5, and smoking status in Table 6.

TABLE 4: PATIENT AGE

	FLUCONAZOLE			AMPHOTERICIN		
	Male	Female	Both	Male	Female	Both
n	8	21	29	8	22	30
mean age (yrs)	53	48	50	52	63	60
std. dev.	18	13	14	20	12	15
minimum	22	26	22	24	48	24
maximum	77	72	77	75	81	81

TABLE 5: AGE GROUPS

	FLUCONAZOLE			AMPHOTERICIN		
	Male	Female	Both	Male	Female	Both
Age (years)						
Under 40	2	5	7	3	0	3
40-49	1	8	9	0	5	5
50-65	3	6	9	2	9	11
Over 65	2	2	4	3	8	11
Total	8	21	29	8	22	30

TABLE 6: SMOKING STATUS

	FLUCONAZOLE			AMPHOTERICIN		
	Male	Female	Both	Male	Female	Both
Non-smoker	1	8	9	2	16	18
Ex-smoker	3	2	5	1	1	2
Smoker	4	11	15	5	5	10
Total	8	21	29	8	22	30

Very few patients had significant concomitant medical conditions. Only two patients in each treatment group were receiving other drug therapy, with one amphotericin patient receiving six additional medications. Only one patient, whose trial drug was fluconazole, began concomitant non-study medication during the trial.

2. Dental Assessment

Of the 59 patients entered into the trial 33 were edentulous in the maxilla. There were 19 such patients in the amphotericin group and 14 in the fluconazole group. The denture wearing status in shown in Table 7.

TABLE 7: TYPE OF DENTURE WORN

	Fluconazole	Amphotericin	Both Groups
CU/CL	8	13	21
CU/PL	0	4	4
CU/-	6	2	8
PU/PL	4	1	5
PU/-	11	10	21

CU=complete upper, CL=complete lower, PU=partial upper, PL=partial lower, -=no lower denture.

The age of the dentures was, in many cases, an approximation. Thirteen patients were unable to estimate the age of their dentures. For both treatment groups, the patients had been wearing their existing dentures for an average of 9 to 10 years, the minumum being less than one year and the maximum being more than 40 years.

The quality of the dentures being worn is indicated by the data in Tables 8 to 13.

TABLE 8: DENTURE QUALITY - RETENTION

	Fluconazole	Amphotericin	Both Groups
Good	7	8	15
Fair	10	10	20
Poor	12	12	24
Total	29	30	59

TABLE 9: DENTURE QUALITY - STABILITY

	Fluconazole	Amphotericin	Both Groups
Good	11	7	18
Fair	7	9	16
Poor	11	14	25
Total	29	30	59

TABLE 10: DENTURE QUALITY - EXTENSION

	Fluconazole	Amphotericin	Both Groups
Good	8	11	19
Fair	11	11	22
Poor	10	7	17
Total	29	29	58

(This assessment was considered not applicable for one patient in the amphotericin group.)

TABLE 11: DENTURE QUALITY - HYGIENE

	Fluconazole	Amphotericin	Both Groups
Good	10	6	16
Fair	15	17	32
Poor	4	7	11
Total	29	30	59

TABLE 12: DENTURE QUALITY - OCCLUSION

	Fluconazole	Amphotericin	Both Groups
Good	10	7	17
Fair	6	9	15
Poor	12	11	23
Total	28	27	55

(This assessment was considered not applicable for one patient in the fluconazole group and three patients in the amphotericin group.)

TABLE 13: DENTURE QUALITY - VERTICAL DIMENSION

	Fluconazole	Amphotericin	Both Groups
Reduced	6	5	11
Correct	21	14	35
Increased	0	2	2
Total	27	21	48

(This assessment was considered not applicable for two patients in the fluconazole group and nine patients in the amphotericin group.)

There is very little difference between treatment groups as far as the individual denture factors are concerned. The information gathered can be presented in a different manner, to indicate the overall quality of each patients denture. For each patient, therefore, a score is given for the denture factors of retention, stability, extension, hygiene and occlusion: good = 1, fair = 2, poor = 3. A denture which was assessed as good for all factors would score the minimum total of 5, whilst a denture that was assessed as poor for all factors would score the maximum total of 15. A score of 10 could be said to represent a denture which was fair overall. A summary of the scores obtained in this manner is shown in Table 14 which shows the number of patients in each

treatment group, and in total, against the scores obtained. Percentage numbers are displayed in parentheses.

TABLE 14: OVERALL DENTURE QUALITY

Score	Fluconazole	Amphotericin	Both Groups
5-9	10(36)	8(31)	18(33)
10	5(18)	4(15)	9(17)
11-15	13(46)	14(54)	27(50)
Total	28	26	54

Patients were excluded from this analysis if any assessment was deemed to be inapplicable, as no score could be given in such an instance. Vertical dimension was not included in the scoring because of the number of "not applicable" cases. It is apparent that the groups are quite evenly matched as far as overall denture quality is concerned. There are more dentures on the poor side of fair than on the good side of fair. If both groups are considered together exactly two thirds of patients had dentures which scored 10 or more.

Summary: denture quality tended towards poor and this is reflected particularly in the factors retention, stability and occlusion.

3. Denture Stomatitis.

The number of patients in each treatment group with each classification of denture stomatitis at the initial examination is given in Table 15.

TABLE 15: DENTURE STOMATITIS - CLASSIFICATION

	Fluconazole	Amphotericin
Localised Erythematous	7	7
Diffuse Erythematous	15	17
Hyperplastic/ granular	7	6
Total	29	30

The treatment groups were well matched in terms of the frequency of each type of denture stomatitis. In total, slightly more than one half of the patients had the diffuse erythematous form of denture stomatitis. The remaining patients were divided almost equally between the localised and the hyperplastic form of the disease. Only nine (5 fluconazole and 4 amphotericin) patients were able to provide even a rough estimate of the

duration of their present episode of denture stomatitis. One patient in each group reported a previous episode of denture stomatitis during the past year. Many patients had no awareness of the condition.

There was a marked difference between smokers and non-somkers with regard to the initial classification of denture stomatitis. Overall there were 25 smokers and 27 non-smokers. Amongst smokers, the hyperplastic granular form of denture stomatitis was the commonest. In fact, of the 13 patients who had hyperplastic granular denture stomatitis, 10 were smokers. Amongst the non-smokers the diffuse erythematous form of denture stomatitis was the most common. Twice as many non-smokers as smokers had this form of the disease. The localised erythematous type was equally distributed amongst smokers and non-smokers.

Nine patients in the fluconazole group and 10 in the amphotericin group had been previously prescribed antifungal drugs. Some patients had received more than one drug in the past and a total of 28 previous antifungal prescriptions were recorded. Twenty four of these prescriptions were for polyenes, with amphotericin and nystatin being equally popular.

4. Baseline Symptoms

On questioning, thirty one patients (52.5%) complained of subjective symptoms of either pain, burning sensations, abnormal tastes or dry mouth, with 14 of these patients complaining of more than one symptom. The commonest complaint was of dry mouth (19 patients), followed by burning sensations (15 patients), abnormal tastes (11 patients) and pain (7 patients). However the majority of symptoms were reported to be mild. Table 16 lists the number of patients with each symptom, according to severity. Of the 31 symptomatic patients 16 were subsequently assigned to receive fluconazole and 15 to receive amphotericin.

TABLE 16: BASELINE SYMPTOMS

	Absent	Mild	Moderate	Severe
Pain	52	3	3	1
Burning Sensation	44	6	6	3
Abnormal Taste	48	6	3	2
Dry Mouth	40	13	3	3

Of the 31 patients with symptoms, 17 had the diffuse erythematous form of denture stomatitis, nine had the hyperplastic form and five had the localised form. Symptoms were therefore commonest amongst patients with hyperplastic/granular denture stomatitis. Approximately 69% of this group of patients had symptoms compared with approximately 53% of those with diffuse erythematous and 36% of those with localised erythematous denture stomatitis.

5. Concomitant Oral Candidoses

Although the term concomitant oral candidoses is used, the diagnosis was made on clinical grounds alone. At the initial assessment there were no patients with acute erythematous candidosis, median rhomboid glossitis or chronic mucocutaneous candidosis. One patient had acute psuedomembranous candidosis and two had chronic hyperplastic candidosis. Eleven patients (18.6%) had angular cheilitis which was bilateral in each case.

6. Efficacy Analyses - Patient Evaluability

Although nine patients did not complete the study according to the protocol, only three were excluded from all efficacy analyses: two amphotericin patients for poor compliance and one fluconazole patient who failed to return after the baseline visit. One further amphotericin patient had negative baseline mycology and was therefore excluded from the mycologic analysis. However this patient responded to treatment and was included in the analysis of clinical efficacy. The most frequent protocol violation was a failure to complete all vists. The timing of the review vists is described in the trial design. In practice there was some variance from this if patients found it necessary to change appointment dates. Some patients were excluded from tables and analyses for particular visits because they attended outside the defined window for that visit. The visit windows are shown in Table 17. Essentially the visit windows were designed to allow patients to be assessed during treatment, after the completion of treatment and at a long term follow-up.

TABLE 17: VISIT WINDOWS

	Days post baseline visit	
	Fluconazole	Amphotericin
Visit		
2 (On treatment)	6-14	6-27
3 (Off treatment)	15-42	28-42
4 (Long term follow-up)	>42	>42

It should be stressed that most patients fell within these limits and were assessed at the time anticipated in the trial design.

7. Clinical Efficacy of Fluconazole and Amphotericin.

(i) Palatal Erythema

Since palatal erythema is the major clinical sign of denture stomatitis this was used as the main indicator of clinical efficacy. Table 18 shows the number of patients in each group who were cured, improved or unchanged at each visit. The chi-square test was used to compare the two groups. There was no significant difference between the two treatments at any visit. (Significance level $p = 0.05$)

TABLE 18: CLINICAL EFFICACY

	1 Week		4 Weeks		12 Weeks	
	F	A	F	A	F	A
Cured	5	9	11	14	6	5
Improved	8	6	10	6	9	7
No Change	13	11	4	2	11	12
Total	26	26	25	22	26	24

(F=fluconazole, A=amphotericin. Cured, improved and no change are in comparison with baseline assessment.)

The best clinical cure rate was seen at the four week visit with approximately 44% of patients in the fluconazole group cured and 63% in the amphotericin group cured. If the categories cured and improved are combined greater clinical efficacy is observed. Table 19 gives the percentage number of patients in each group who were either cured or improved at each visit, in comparison with the baseline assessment.

TABLE 19: CURED OR IMPROVED

	1 Week	4 Weeks	12 Weeks
Fluconazole	50%	84%	58%
Amphotericin	58%	91%	50%

Both drugs had achieved their maximum observed efficacy at the one month visit. At this visit the 95% confidence limits are (70%,98%) for fluconazole and (79%, 100%) for amphotericin. It is therefore 95% certain that the cure rate lies between 70% and 98% for fluconazole and between 79% and 100% for amphotericin. By the time of the long term follow-up there had clearly been considerable relapse and recurrence, with figures for patients cured or improved returning towards the level seen after one week of treatment.

(ii) Symptoms

At all visits patients were asked to grade the symptoms pain, burning sensation, abnormal taste and dry mouth, as absent, mild, moderate or severe. The results for each symptom at each follow-up visit were crosstabulated against baseline symptom severity. Table 20 is an example of such a crosstabulation. The complete set of these tables may be found in Appendix 2.

TABLE 20: SYMPTOM - BURNING SENSATION, 1 WEEK VISIT

	FLUCONAZOLE		AMPHOTERICIN		
	Absent	Mild	Absent	Mild	Moderate
BASELINE					
Absent	19	0	18	1	0
Mild	3	1	1	1	0
Moderate	1	0	0	0	3
Severe	2	0	0	1	0

It is apparent that 19 patients in the fluconazole group who had no burning sensation at the baseline assessment continued to have an absence of this symptom after one week. This symptom was also absent for three fluconazole patients who had mild baseline severity, for one who had moderate and for two who had severe symptoms of burning at the baseline visit. One fluconazole patient who had mild burning sensations at the baseline assessment continued to have mild symptoms after one week. Such comparisons can also be made for the amphotericin group.

For statistical purposes the responses were scored as absent=0, mild=1, moderate=2 and severe=3. From Table 20 it can be seen that the total number of evaluable patients in the fluconazole group at one week is 26. It

is possible to deduce from the figures that the combined baseline score for these patients, for the symptom burning sensation, was 12. However at one week the combined score had fallen to one, a reduction of 11.

The change in score from the baseline to the one week visit was calculated for each symptom and the results added together to give the change in *total* symptom score. This calculation was repeated for the four week and the 12 week visits. Table 21 shows the mean change, from the baseline, in the total symptom score at each visit, for both treatment groups.

TABLE 21: MEAN CHANGE FROM BASELINE IN TOTAL SYMPTOM SCORE

	1 Week	4 Weeks	12 Weeks
Fluconazole	1.08(n=26)	1.24(n=25)	0.69(n=26)
Amphotericin	0.2 (n=25)	0.57(n=21)	0.52(n=23)

In each case the change is a reduction in score from the baseline. Although the reduction in total symptom score was consistently greater in the fluconazole group the difference between treatments was not significant at any visit (t test). The greatest reduction in symptom score was observed at the four week visit.

The mean changes in total symptom score appear to be of a rather small magnitude. It should be recalled that the total number of evaluable patients at each visit, used for the calculation of the mean, included many patients who had no symptoms.

Given the way in which the symptoms were graded it would clearly be possible to recognise when a patients' symptoms had been cured or improved, in comparison with the baseline assessment. Table 22 gives the percentage number of patients at each follow-up visit, whose symptoms were either cured or improved in comparison with the baseline assessment.

TABLE 22: SYMPTOMS CURED OR IMPROVED (%)

	1 Week		4 Weeks		12 Weeks	
	F	A	F	A	F	A
Pain	100	100	75	100	33	50
Burning	86	29	100	43	66	57
Ab. Taste	62	25	62	66	66	33
Dry Mouth	57	36	87	50	85	60

(F=fluconazole, A=amphotericin)

In each case the percentage figure is based on the total number of patients at each visit who were symptomatic, or who had been symptomatic at the baseline assessment. On the whole the greatest efficacy was observed at the four week visit when the majority of patients demonstrated cured or improved symptoms. Pain and burning sensation were generally more amenable to treatment than abnormal taste and dry mouth, although the effect on pain appears not to have been sustained as long as the effect on other symptoms.

(iii) Concomitant Oral Candidoses

One patient in the fluconazole group had acute pseudomembranous candidosis at the baseline assessment. This had resolved by the one week visit but by the 12 week visit it had recurred. One patient in the amphotericin group had developed acute psuedomembranous candidosis at the 12 week visit, this condition having been absent at all previous visits. Two fluconazole patients had chronic hyperplastic candidosis at the baseline assessment, both were cured by the four week visit and remained cured at the long term follow-up.

Table 23 shows the number of patients in each treatment group with angular cheilitis, at each visit. (Angular cheilitis was bilateral in every case.)

TABLE 23: ANGULAR CHEILITIS

	Baseline	1 Week	4 Weeks	12 Weeks
Fluconazole	7	5	2	4
Amphotericin	4	3	1	1
Both Groups	11	8	3	5

The figures in Table 23 represent the same group of patients progressing through visits, so it is possible to conclude that of the seven fluconazole patients with angular cheilitis two were cured by week one, five by week four, and so on. The numbers are small so statistical comparisons were not performed. However the results in each treatment group appear similar. Of those patients who had angular cheilitis at the baseline almost three quarters were clear of the condition by one month. Recurrence of angular cheilitis by 12 weeks appears to have been more of a problem in the fluconazole group.

Summary: there was no detectable difference between fluconazole and amphotericin in respect of any measure of clinical efficacy.

8. Mycologic Efficacy of Fluconazole and Amphotericin.

If no yeast organisms were cultivated from the swabs or oral rinse at the follow-up visits, the patient was deemed to be mycologically cured. In patients not rendered yeast free it was felt that the most important indicator of mycologic improvement would be a decrease in the density of yeast colonisation within the patients' mouth. This would be reflected in a reduction in the colony count derived from the oral rinse sample. Colony counts varied considerably between patients and an absolute figure representative of improvement could not be identified. It was decided, therefore, to look at the figures for each patient in turn and to set an arbitrary level of 50% reduction in colony count, from the baseline, as the measure of improvement at each follow-up visit. Table 24 details the mycologic response to treatment, showing the number of patients cured, improved and unchanged at each visit.

More amphotericin than fluconazole patients were mycologically cured at both the one week and four week visits. The difference between treatments at four weeks is statistically significant ($p<0.01$ chi-square).

TABLE 24: MYCOLOGIC EFFICACY

	1 Week		4 Weeks		12 Weeks	
	F	A	F	A	F	A
Cured	8	12	3	10	4	1
Improved	5	5	2	2	0	8
No Change	13	8	20	9	22	14
Total	26	25	25	21	26	23

(F=fluconazole, A=amphotericin. Cured, improved and no change are in comparison with the baseline assessment.)

The significantly better cure rates for amphotericin at four weeks are unsurprising, given that at this stage the amphotericin group had just completed treatment, whereas the fluconazole group had completed their treatment 14 days previously. This difference is also reflected in the combined figures for cure and improvement, expressed in table 25 in percentage terms.

TABLE 25: MYCOLOGIC CURE OR IMPROVEMENT

	1 Week	4 Weeks	12 Weeks
Fluconazole	50%	20%	15%
Amphotericin	68%	57%	39%

Tables 24 and 25 illustrate the difficulty of eradicating yeasts from the mouth with antifungal therapy. The best mycologic response occurred at one week following which there was a gradual deterioration. This is well illustrated by Figure 1. No more than half the patients were cured in either treatment group at any visit. There is an apparent anomaly in the fluconazole group (Table 24), in that the number of cured patients appears to rise from three to four in the period between the four and 12 week visits. However, it should be recalled that the number of evaluable cases differed from visit to visit and there were, therefore, slight differences in the patient cohorts at each visit.

Summary: the mycologic response was more pronounced and more prolonged in the amphotericin group. However the course of amphotericin treatment was twice as long as the course of fluconazole treatment. Initial reduction in yeast colonisation in both groups was followed by repopulation after the cessation of treatment.

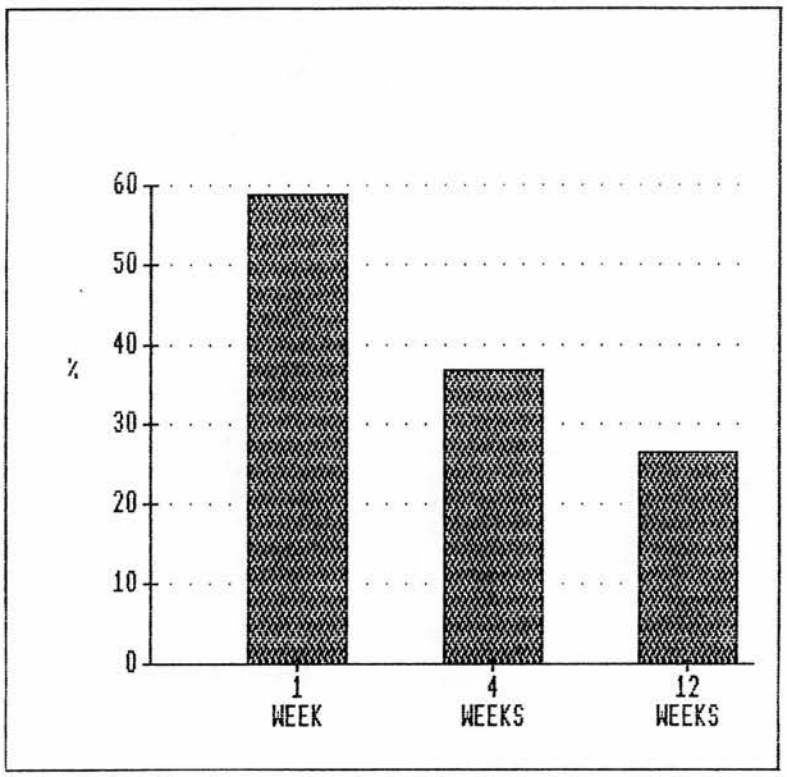

Figure 1. Mycologic cure/improvement, all patients.

9. Correlation of Clinical and Mycologic Response

Whereas the best mycologic response was observed at one week the best clinical response was seen at four weeks. By four weeks the mycologic cure and improvement rate had begun to decline and this was true even in the amphotericin group who had only recently completed treatment. At the 12 week visit there had been considerable clinical relapse and recurrence and the decline in mycologic status continued (Figures 2 and 3). The clinical response appeared to lag behind the mycologic response.

When the clinical and mycologic status of each patient were compared at each follow-up visit, there was little correlation. The hypothesis was that mycologic improvement, based on a 50% or more reduction in colony count, derived from the oral rinse, should be reflected in clinical cure or improvement. No such reduction in colony count should be reflected in an unchanged clinical status with respect to the baseline. At the four week visit correlation between mycologic and clinical response was observed in only 30% of fluconazole patients and in 60% of amphotericin patients.

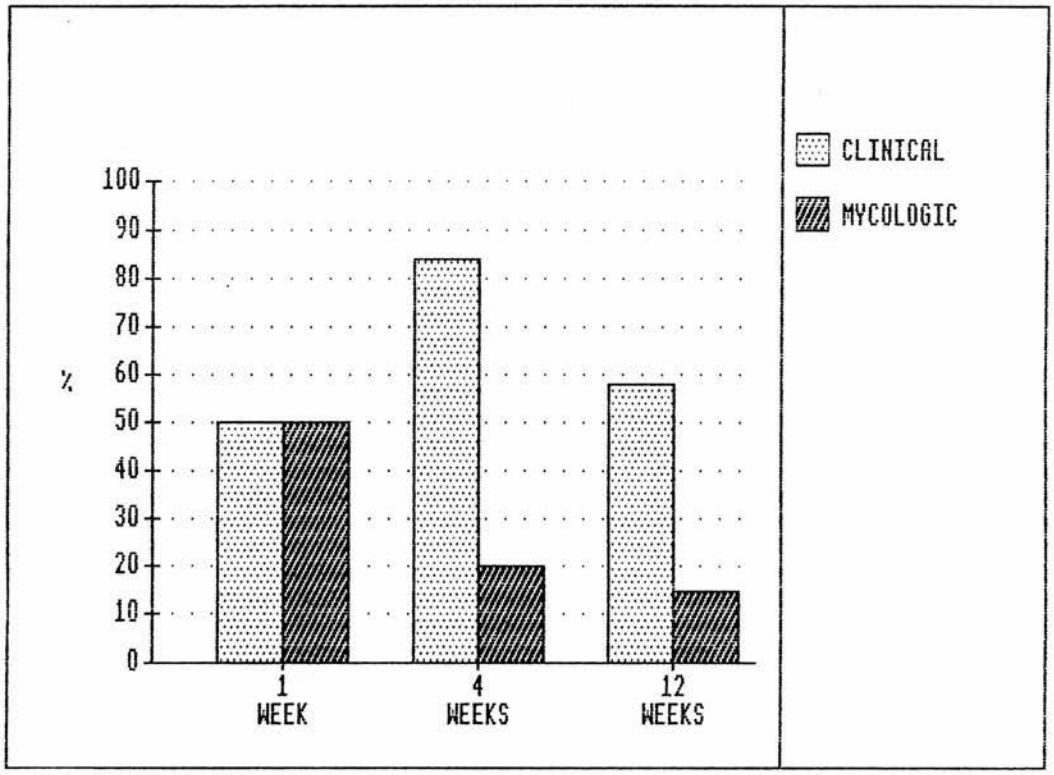

Figure 2. Clinical and mycologic cure/improvement, fluconazole group.

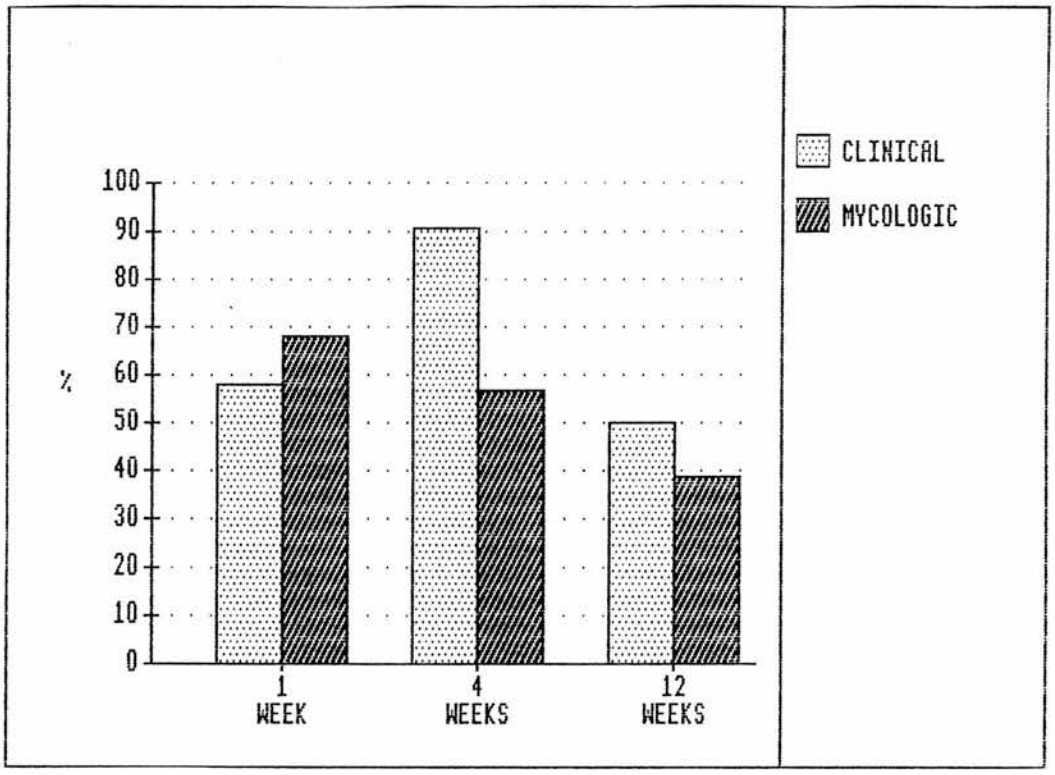

Figure 3. Clinical and mycologic cure/improvement, amphotericin group.

If clinical response truly lagged behind mycologic response it might be possible to predict from each patients mycologic status, what their clinical status would be at the succeeding follow-up visit. The data for each patient were examined to see if the mycologic status at the one week visit could be used to predict the clinical status at the four week visit and also if the mycology at four weeks was predictive of the clinical response at 12 weeks. A mycologic cure or improvement at week one should then indicate that the patient would be clinically cured or improved at week four, whereas an unchanged mycologic status at week one should indicate an unchanged clinical status at week four, and so on.

Such predictions proved to be correct on only 47% of occasions for fluconazole patients, and on 56% of occasions for amphotericin patients. This poor correlation between clinical and mycologic response requires explanation. Mycologic cure and improvement never reached the highest levels of clinical cure and improvement. Judgement of clinical improvement is, by its nature, subjective and imprecise. It may also be, that the criteria adopted to indicate mycologic improvement were inappropriate. In addition the oral rinse technique may not be the most suitable for the quantitation of yeasts in the mouths of denture wearers. The denture fitting surface is believed to be the most important site

of yeast colonisation in these patients and even if the denture is in the mouth when the rinse is performed, the fitting surface may not be accessible.

It may be that the presence or absence of yeasts grown from swabs of various oral sites is more relevant to the clinical response. Yeasts were isolated from the mouths of 58 (98%) of the 59 patients who entered the study. At the baseline assessment, yeasts were cultivated from the swabs of six oral sites as follows:

Palate	-	91.5% of all patients
Denture	-	88%
Tongue	-	90%
Floor of mouth	-	73%
Right angle	-	46%
Left angle	-	40%

It can be seen that yeasts were isolated most frequently from the palate, tongue and the fitting surface of the upper denture. At follow-up visits, at least during the period of treatment, the frequency of isolation could be expected to fall, or looked at in the opposite way, absence of growth at these sites could be a measure of improvement for the group as a whole. This is illustrated in Figure 4.

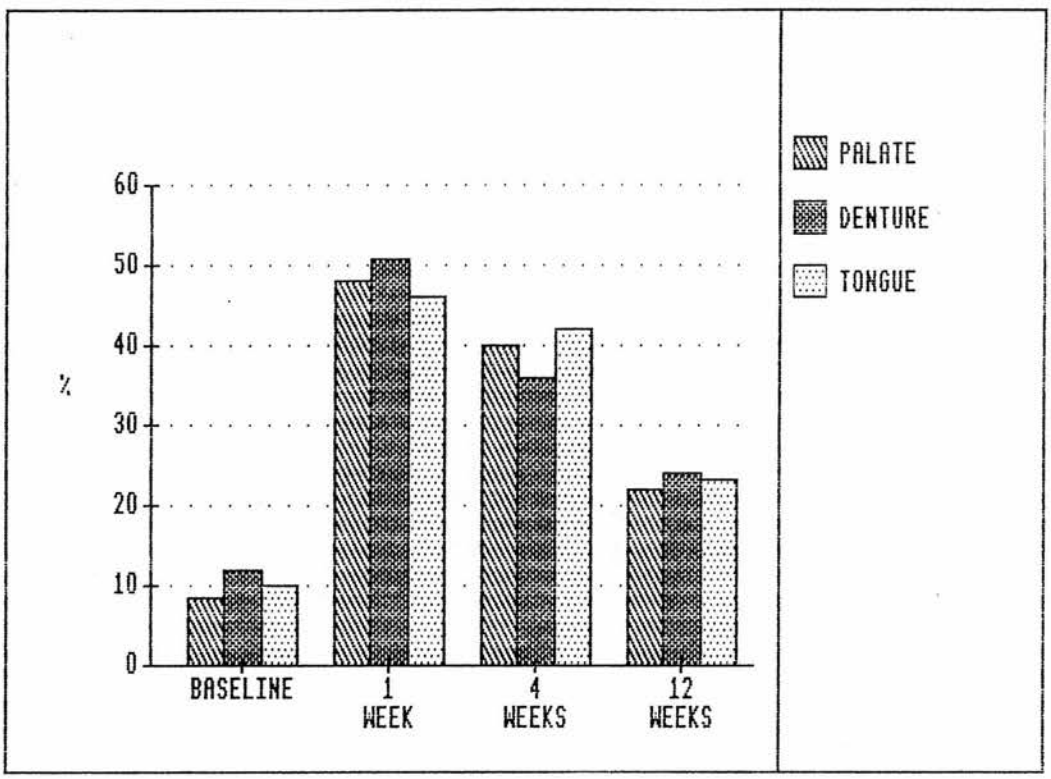

Figure 4. Percentage of all patients with no yeast growth derived from palate, denture or tongue.

At the baseline very few patients had no growth derived from the palate, denture or tongue. The best mycologic response is observed at one week, when no yeasts were cultivated from these sites in approximately half of all patients. The percentage of patients who improved with respect to yeast growth at these sites is very similar to that for mycologic cure and improvement based on reduction in oral rinse colony count. Again the best mycologic improvement observed does not approach the level of the best clinical response.

Using yeast growth from the palate or denture fitting surface as a measure, the correlation between mycologic and clinical response for the individual patient was again examined. The hypothesis was that negative culture from one or both of these sites would represent mycologic improvement and that this should be reflected in clinical cure or improvement. Positive culture from both sites would be reflected in an unchanged clinical status with respect to the baseline.

When mycologic and clinical results were compared in this way, for each patient at the four week visit, correlation was observed in 52% of fluconazole patients and in 73% of amphotericin patients. If the same measure of mycologic response was used in an attempt to predict the clinical response, as previously described for oral rinse colony

count, predictions were correct on 55% of occasions for fluconazole patients and on 66% of occasions for amphotericin patients.

It would appear as though absence of yeast growth from either the palate or denture, or both, may be a more relevant indicator of mycologic improvement than 50% reduction in colony count, as derived from an oral rinse. The slightly better correlation between clinical and mycologic response in the amphotericin group may relate to the length of treatment, the timing of the follow-up visits and the local application of the drug.

Finally, in this section, it is revealing to look at mycologic events in those patients who showed an excellent clinical response and in those whose clinical response was very poor. An excellent response was defined as cure which was sustained throughout the study and a very poor response was defined as an unchanged clinical status persisting throughout the study. Eight patients (three fluconazole, five amphotericin) showed an excellent response. In three of these, yeasts were completely eliminated at the one and four week visits. In a further four patients there was a dramatic and sustained reduction in the number of sites at which growth occurred and in colony counts. Six patients (four fluconazole, two amphotericin) showed a very poor

clinical response. All six patients showed high rates of yeast recovery throughout the study.

Summary: mycologic improvement based on reduction in colony count, derived from an oral rinse, did not correlate closely with clinical events. Positive or negative yeast culture from the denture, palate, or both, seemed more pertinent. At the extreme ends of the spectrum of clinical success the correlation with mycologic events was clearer.

10. Clinical Outcome in each Classification of Denture Stomatitis.

The effect of the initial classification of denture stomatitis on clinical response at each follow-up visit was examined. Table 26 shows the number of patients with each classification of denture stomatitis who were either cured or improved, or unchanged. Patients from both treatment groups were combined for this analysis. Cure and improvement for each classification is shown in percentage terms in Table 27.

TABLE 26: DENTURE STOMATITIS CLASSIFICATION Vs CLINICAL RESPONSE

	1 Week			4 Weeks			12 Weeks		
	D	H	L	D	H	L	D	H	L
Cured/Improved	18	5	7	26	9	10	16	4	4
No Change	13	6	5	4	2	2	13	5	8
Total	31	11	12	30	11	12	29	9	12

(D=Diffuse, H=Hyperplastic, L=Localised)

TABLE 27: CURE/IMPROVEMENT IN DIFFUSE, HYPERPLASTIC AND LOCALISED DENTURE STOMATITIS

	1 Week	4 Weeks	12 Weeks
Diffuse	58%	87%	55%
Hyperplastic	45%	82%	44%
Localised	58%	83%	33%

The clinical response follows the same pattern as previously observed. Patients appeared to respond equally well to antifungal treatment, whatever their initial classification of denture stomatitis had been. Analysis using the chi-square test showed no significant association between denture stomatitis classification and cure or improvement.

11. Clinical Efficacy. The effect of sex and smoking.

Since the clinical response was similar in both treatment groups all the patients will be considered together to study the effect of various patient and prosthetic factors.

(i) Patient Sex.

Table 28 shows the number of males and females against clinical response, with respect to the baseline assessment, at each follow-up visit. Table 29 shows the percentage of males and females who were clinically cured or improved at each follow-up visit.

TABLE 28: SEX vs CLINICAL RESPONSE

	1 WEEK		4 WEEKS		12 WEEKS	
	M	F	M	F	M	F
Cured/Improved	5	23	10	31	5	22
No Change	11	13	2	4	10	13
Total	16	36	12	35	15	35

(M=Male, F=Female)

TABLE 29: CURE/IMPROVEMENT IN MALES AND FEMALES

	1 WEEK	4 WEEKS	12 WEEKS
MALE	31%	83%	33%
FEMALE	64%	89%	63%

Women appeared to respond consistently better than men. The difference at one week was statistically significant ($p<0.05$, chi-square). There was no significant difference by four weeks and the difference at 12 weeks just failed to reach significane at the 5% level.

(ii) Smoking status.

Table 30 shows the number of smokers, ex-smokers and non-smokers, against clinical response, at each follow-up visit,in comparison with the baseline. Table 31 shows the percentage of smokers, ex-smokers and non-smokers who were cured or improved at each follow-up visit.

TABLE 30: SMOKING STATUS vs CLINICAL RESPONSE

	1 WEEK			4 WEEKS			12 WEEKS		
	S	XS	NS	S	XS	NS	S	XS	NS
Cured/Improved	8	3	17	16	6	19	12	3	12
No Change	13	4	7	3	1	2	11	4	8
Total	21	7	24	19	7	21	23	7	20

(S=Smoker, XS=Ex-Smoker, NS=Non-Smoker)

TABLE 31: CURE/IMPROVEMENT IN SMOKERS, EX-SMOKERS AND NON-SMOKERS

	1 WEEK	4 WEEKS	12 WEEKS
SMOKERS	38%	84%	52%
EX-SMOKERS	43%	86%	43%
NON-SMOKERS	71%	90%	60%

Non-smokers seemed to respond better than smokers and this difference was most marked at one week. The association between cure or improvement and smoking status at one week just failed to reach significance at the 5% level using the chi-square test. However if ex-smokers are excluded from the analysis the difference in cure and improvement rates between non-smokers and

smokers is significant ($p<0.05$, chi-square). The difference at four and 12 weeks was not significant. This implies that the initial response of denture stomatitis to antifungal treatment is slower in smokers than in non-smokers.

If this is the case it is interesting to note that whilst 56% of women were non-smokers, only 19% of men were non-smokers. In fact the association between sex and smoking status was significant ($p<0.05$, chi-square). This may explain, at least partially, why women showed a better clinical response than men.

12. Denture Wearing Habits

Patients were advised at the begining of the trial to leave their dentures out at night. Compliance with such instructions is difficult to estimate. A few weeks after completion of the trial patients were sent a letter containing two questions as follows:

1. Before you began the treatment did you leave your dentures in at night? YES/NO

2. Do you now leave your dentures in at night? YES/NO

A stamped addressed envelope was provided for reply.

It was felt that if these questions were asked after completion of the trial patients would be more inclined to answer honestly. Forty six of the 59 patients replied, 22 of whom had received fluconazole and 24 of whom had received amphotericin. Of these 46 patients, 27 said that before the trial began they had worn their dentures at night (13 fluconazole, 14 amphotericin). Fourteen said that they now wore their dentures at night (8 fluconazole, 6 amphotericin). Therefore, if the responses are to be believed, 13 of 27 patients who had originally worn their dentures at night had followed instructions and were now leaving their dentures out at night.

Patients who wear their dentures at night can be considered as more or less constant denture wearers, as opposed to day-time only wearers. The effect of constant denture wearing on cure and improvement rates was examined. It was assumed that the patients' responses to the questionaire were truthful and their response to the second question was taken to indicate whether they were constant or day-time only denture wearers at the time of the trial. Table 32 shows the number of constant and day-time denture wearers who were cured or improved, or unchanged, at each visit.

TABLE 32: DENTURE WEARING vs CLINICAL RESPONSE

	1 WEEK		4 WEEKS		12 WEEKS	
	C	D	C	D	C	D
Cured/Improved	10	14	9	25	7	15
No Change	4	15	2	3	5	16
Total	14	29	11	28	12	31

(C=Constant denture wearers, D=Day-time denture wearers)

At one week, 71% of constant denture wearers were cured or improved compared with 48% of day-time only wearers. This discrepancy is surprising but it did not continue throughout the study. At four weeks 82% of constant denture wearers were cured or improved compared with 89% of day-time only wearers and at 12 weeks the figures are 58% and 48% respectively. The apparently better early response in constant denture wearers was not statistically significant but is nonetheless intriguing. It might be that it is related to topical application of the drug in amphotericin patients. If the results at one week are broken down into treatment groups, 75% of constant denture wearers taking fluconazole were cured or improved compared with 66% of constant denture wearers taking amphotericin. The trend is therefore opposite to what might be expected if topical drug application combined with constant denture wearing had some benefit.

13. Clinical Efficacy.

The effect of prosthetic factors.

The effect of various prosthetic factors on the clinical response to treatment was evaluated. In these evaluations all patients were considered together, including those who had attended outside the defined follow-up visit windows. It should be recalled that certain of the denture quality assessments were not considered applicable to some patients.

(i) Upper Denture Type

Table 33 shows the number of patients wearing complete upper and partial upper dentures against clinical response at each visit. Percentage figures for cured/improved are given in parentheses.

TABLE 33: UPPER DENTURE TYPE vs CLINICAL RESPONSE

	1 WEEK		4 WEEKS		12 WEEKS	
	C	P	C	P	C	P
Cured/Improved	19(63)	11(45)	28(93)	17(74)	15(53)	12(54)
No Change	11	13	2	6	13	10
Total	30	24	30	23	28	22

(C=Complete denture, P=Partial denture)

Although the figures suggest that complete denture wearers showed a better clinical response than partial denture wearers, the difference was not significant at any visit. One possible explanation for any difference observed might be that the gingival crevice provides an additional reservoir of yeast organisms in partially dentate individuals. If this were so, might fluconazole, secreted in gingival crevicular fluid, prove a more effective drug for the treatment of denture stomatitis in patients with partial dentures? Table 34 shows the number of patients wearing upper partial dentures, in each treatment group, against clinical response at each visit. Percentage figures for cured/improved are given in parentheses. For both Tables 33 and 34 the categories cured and improved are in comparison with the baseline.

TABLE 34: CLINICAL RESPONSE IN UPPER PARTIAL DENTURE WEARERS, FLUCONAZOLE vs AMPHOTERICIN

	1 WEEK		4 WEEKS		12 WEEKS	
	F	A	F	A	F	A
Cured/Improved	6(43)	5(50)	9(64)	8(89)	7(50)	5(62.5)
No Change	8	5	5	1	7	3
Total	14	10	14	9	14	8

(F=Fluconazole, A=Amphotericin)

Although the numbers involved are small, the hypothesis that fluconazole is a better drug for the treatment of denture stomatitis in the partially dentate is not supported.

(ii) Denture Quality

The effect of each of the denture quality factors, assessed at the baseline visit, on clinical outcome was studied. The results are summarised in Tables 35-40. These tables show the percentage number of patients who were cured or improved against the denture quality factor assessment (in Table 35, of those patients who had good denture retention, 64% were cured or improved at one week, 100% at four weeks and 33% at 12 weeks, and so on). Cure/improvement is in comparison with the baseline.

TABLE 35: CURE/IMPROVEMENT vs DENTURE RETENTION

	1 Week	4 Weeks	12 Weeks
Good	64%	100%	33%
Fair	50%	80%	80%
Poor	59%	82%	53%

TABLE 36: CURE/IMPROVEMENT vs DENTURE STABILITY

	1 Week	4 Weeks	12 Weeks
Good	56%	94%	47%
Fair	54%	83%	58%
Poor	61%	83%	60%

TABLE 37: CURE/IMPROVEMENT vs DENTURE EXTENSION

	1 Week	4 Weeks	12 Weeks
Good	61%	95%	42%
Fair	47%	82%	62%
Poor	62%	80%	61%

TABLE 38: CURE/IMPROVEMENT vs DENTURE HYGIENE

	1 Week	4 Weeks	12 Weeks
Good	53%	87%	47%
Fair	59%	89%	60%
Poor	60%	78%	55%

TABLE 39: CURE/IMPROVEMENT vs OCCLUSION

	1 Week	4 Weeks	12 Weeks
Good	43%	75%	21%
Fair	61%	83%	58%
Poor	68%	91%	70%

TABLE 40: CURE/IMPROVEMENT vs VERTICAL DIMENSION

	1 Week	4 Weeks	12 Weeks
Decreased	73%	91%	70%
Correct	60%	76%	47%
Increased	50%	100%	50%

For the most part the individual denture quality factors appear to have had no detectable influence on the clinical response to treatment. In other words, for each factor assessed, patients with good, fair or poor dentures responded equally well to antifungal treatment. This was not true for denture retention and occlusion at the 12 week visit. At this visit there was a significant association ($p<0.05$, chi-square) between denture retention and cure/improvement, patients with fair retention apparently responding better than those with good or poor retention. Also at the 12 week visit, there was a significant association ($p<0.05$, chi-square) between occlusion and cure/improvement. Patients with poor occlusion responded better than those with fair, who in turn responded better than those with good occlusion. (The chi-square test was used where the differences appeared marked, using the actual proportions and not the percentages displayed in the tables.)

Patients with decreased occlusal vertical dimension seemed to show a better response to treatment than those with correct occlusal vertical dimension. However the differences were not significant at any visit. It is useful to recall, when looking at table 40, that there were only two patients in the whole group who had increased vertical dimension.

14. Haematology and Liver Function Tests.

At the baseline assessment, only seven of 59 patients displayed a haematologic deficiency. Five patients (four assigned to fluconazole and one to amphotericin) had a low serum ferritin and two patients (both assigned to receive amphotericin) had a low serum folate. One of the patients with low serum folate was excluded from analysis due to poor compliance with treatment. All of the six remaining patients responded to treatment despite the fact that the deficiencies were not corrected.

During the period of active treatment no adverse changes in haematologic indices or liver function tests were observed.

15. Yeast Species.

Yeasts from all sites sampled were speciated. Yeasts were most frequently isolated from the palate, denture and tongue. Qualitative changes in the yeast flora at these three sites during the period of the study are considered in this section.

Three sites in 59 patients gives a total of 177 sites. The relative frequency of different yeast species at these sites, at the baseline visit, was as follows:

No growth - 10.2%

***Candida albicans* - 69.5%**

***Candida glabrata* - 16.4%**

***Saccharomyces cerevisiae* - 5.1%**

Unidentified - 2.2%

The percentages do not add up to 100 since there was mixed growth (growth of more than one organism) at 3.4% of sites. This level of mixed growth remained much the same throughout the study. At the baseline visit, mixed growth was always a combination of *C. albicans* and *C. glabrata*. At follow-up visits, other combinations were observed.

Changes in the relative frequency of yeast species isolation during the course of the study are shown, for

the fluconazole group in Table 41 and for the amphotericin group in Table 42. The percentages are based on the total number of sites (palate, denture and tongue) sampled for each group at each visit.

TABLE 41: YEAST SPECIES. FLUCONAZOLE GROUP.

	Baseline	1 Week	4 Weeks	12 Weeks
No Growth	9%	36%	25%	21%
C.albicans	71%	39%	55%	55%
C.glabrata	19%	20%	21%	16%
S.cerevisiae	7%	6%	1%	4%
C.parapsilosis	-	3.6%	-	-
Unidentified	-	-	10%	4%

TABLE 42: YEAST SPECIES. AMPHOTERICIN GROUP

	Baseline	1 Week	4 Weeks	12 Weeks
No Growth	11%	61%	52%	24%
C.albicans	68%	27%	38%	64%
C.glabrata	13%	6%	6%	10%
S.cerevisiae	3%	-	-	4%
C.tropicalis	-	-	5%	-
Rhodotorula spp.	-	-	1%	-
Unidentified	4%	9%	1%	4%

At the baseline visit there was little difference between the two treatment groups in terms of the relative frequency of yeast species isolated. The changes also follow a similar pattern in both groups, with no growth being maximal at one week followed by a progressive recolonisation of sites. However amphotericin seemed to be more effective in eliminating yeast growth than fluconazole and this has already been demonstrated in this chapter (see section 8). The increased number of sites at which no growth occurred at follow-up visits, compared with the baseline, seemed to be largely at the expense of *Candida albicans*. The changes in the frequency of this organism seemed to be almost the opposite of the changes in "no growth".

In the fluconazole group the frequency of isolation of *Candida glabrata* remained almost unchanged throughout the study. At the baseline visit a total of 11 patients had *C. glabrata* isolated from one or more of the palate, denture or tongue. Of these 11, six were assigned to receive fluconazole and five to receive amphotericin. Of the six fluconazole patients *C. glabrata* persisted throughout the study in three cases and in two cases it was only eliminated at the final visit. *C. glabrata* was completely eliminated throughout the study in three of the five amphotericin patients. The sustained frequency of isolation of *C. glabrata* in the

fluconazole group can therefore be largely explained by the persistent isolation of the organism from the same patients. *C. glabrata* occurred in only one fluconazole patient at follow-up visits in whom it had not occurred at the baseline.

Of the 11 patients in whom *C. glabrata* had been isolated at the baseline, none failed to attend at any follow-up visit. These patients therefore had a total of 33 follow-up visits. *C. glabrata* was isolated at 18 of these patient visits and on 14 of these visits at all three sites (palate, denture and tongue). Persistance of *C. glabrata* was associated on two occasions with a clinical cure, on six occasions with a clinical improvement and on 10 occasions with an unchanged clinical status in relation to the baseline. Complete elimination of *C. glabrata* was associated on two occasions with clinical cure, on seven occasions with clinical improvement and on six occasions with an unchanged clinical status in relation to the baseline.

Six of the 11 patients in whom *C. glabrata* had been isolated at the baseline, were men. This is a far higher proportion of men than in the experimental group as a whole. Seven of the 11 were smokers and four were non-smokers, although this appears to have had little effect on the mycologic or clinical response in this sub-group of patients.

Other yeast species occurred with varying frequency and in no particular pattern. They usually replaced eliminated *C. albicans* and were almost always associated with improved clinical status.

Summary: Fluconazole and amphotericin were both effective in reducing the frequency of isolation of *Candida albicans*, although amphotericn was more so. *Candida glabrata* seemed relatively resistant to fluconazole.

16. Humoral Immune Response.

The data for almost all of the estimations of immunoglobulin concentration were markedly asymmetrical. The median is therefore a better measure of the centrality of the distribution of the data than the mean. The median values for total, isotype specific, salivary and serum immunoglobulin concentrations are given in Tables 43 and 44.

TABLE 43: MEDIAN VALUES OF TOTAL SALIVARY IMMUNOGLOBULIN LEVELS (IU/litre)

	Baseline		1 Week		4 Weeks		12 Weeks	
	F	A	F	A	F	A	F	A
IgG	0.03	0.31	0.0	0.19	0.0	0.0	0.28	0.32
IgA	5.6	5.2	4.85	4.9	5.1	4.72	5.5	5.27
IgM	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

(F=fluconazole, A=amphotericin)

TABLE 44: MEDIAN VALUES OF TOTAL SERUM IMMUNOGLOBULIN LEVELS (IU/ml)

	Baseline		1 Week		4 Weeks		12 Weeks	
	F	A	F	A	F	A	F	A
IgG	211	243	203	227	203	211	195	227
IgA	26	34	26	32	25	31	27	28
IgM	37	24	36	33	34	30	37	32

(F=fluconazole, A=amphotericin)

The median values for salivary and serum anti-candidal antibody concentrations are given in Tables 45 and 46.

TABLE 45: MEDIAN VALUES OF SALIVARY ANTI-CANDIDAL IMMUNOGLOBULIN LEVELS (ELISA units)

	Baseline		1 Week		4 Weeks		12 Weeks	
	F	A	F	A	F	A	F	A
IgG	4.91	3.56	2.4	2.77	2.4	4.0	2.66	3.32
IgA	62.36	44.57	58.31	44.64	49.94	58.44	61.9	49.07
IgM	9.74	9.00	10.69	8.99	11.33	7.95	7.5	8.52

TABLE 46: MEDIAN VALUES OF SERUM ANTI-CANDIDAL IMMUNOGLOBULIN LEVELS (ELISA units)

	Baseline		1 Week		4 Weeks		12 Weeks	
	F	A	F	A	F	A	F	A
IgG	149	304	240	362	184	264	136	276
IgA	48	20	47	15	36	56	29	66
IgM	301	54	192	123	100	103	100	102

The range of concentrations observed for a given immunoglobulin at a particular visit was often considerable. For this reason relatively small changes in median immunoglobulin concentrations over the course of the study should be interpreted with caution. With this in mind it is possible to make the following observations.

Immunoglobulin concentrations were generally higher in serum than in saliva, with the exception of anti-candidal IgA, which was higher in saliva. In serum, IgG was the predominant isotype. There were similar levels of total serum IgA and IgM. In saliva, IgA was by far the predominant isotype. Total salivary IgG was present in slightly higher levels than total salivary IgM.

Total salivary immunoglobulin levels tended to fall slightly in response to treatment and then rise after the completion of treatment. Total serum immunoglobulin levels tended to remain unchanged throughout the study.

Salivary anti-candidal IgG fell in the fluconazole group after the baseline visit and then remained at a fairly constant level whereas in the amphotericin group it had fallen by one week and then rose again subsequently. A similar falling then rising pattern was observed for salivary anti-candidal IgA in the fluconazole group. However in the amphotericin group, salivary anti-candidal IgA remained quite constant. Salivary anti-candidal IgM levels also tended to remain constant.

By week one serum anti-candidal IgG had risen from baseline levels for both treatment groups but by four weeks it had fallen, the concentration in the amphotericin group being less than at the baseline. In the fluconazole group, serum anti-candidal IgA and IgM both fell after the baseline visit and failed to rise after the completion of treatment. In the amphotericin group serum anti-candidal IgA had fallen by one week but then rose to levels higher than at the baseline.

The most frequently observed pattern of change then, was a fall in immunoglobulin levels at some stage during the treatment period. This was sometimes followed by a rise in immunoglobulin levels after treatment had ceased. This seemed to be more apparent in salivary than in serum immunoglobulins. It must be re-emphasised that the changes in the median values were small and the range of values, in most cases, wide.

Differences were observed in the humoral immune response of smokers and non-smokers. Immunoglobulin levels, particularly in serum, were frequently higher in non-smokers than in smokers. The following total and anti-candidal immunoglobulins were present in significantly higher concentrations in non-smokers than smokers:

Baseline - Total serum IgG, $p<0.01$
Serum anti-candidal IgG, $p<0.01$
1 Week - Total serum IgG, $p<0.05$
Total serum IgA, $p<0.05$
Serum anti-candidal IgG, $p<0.01$
4 Weeks - Total serum IgA, $p<0.05$
Serum anti-candidal IgG, $p<0.01$
12 Weeks - Total serum IgA, $p<0.05$
Serum anti-candidal IgG, $p<0.001$

The Mann-Whitney and Wilcoxon rank sum non-parametric tests of significance were used. As can be seen, significant differences between smokers and non-smokers were observed in serum but not in salivary immunoglobulin levels.

Summary: immunoglobulin concentrations in serum and saliva varied widely between patients and showed relatively small changes in response to antifungal treatment. The humoral immune response in smokers seemed to be depressed in comparison with non-smokers.

17. Adverse Reactions to Treatment.

Adverse reactions to treatment were rare. Only three of the 29 fluconazole patients and five of the 30 amphotericin patients experienced adverse events related or possibly related to treatment. One fluconazole and two amphotericin patients reported the adverse reaction as severe. The single fluconazole patient developed pharyngitis which was tolerated. Of the two amphotericin patients with severe adverse reactions, one developed a burning mouth and the other complained of nausea. This last patient permanently discontinued treatment as a result. In all, three patients using amphotericin complained of nausea.

CHAPTER SIX

A PRELIMINARY STUDY OF THE CELLULAR IMMUNE RESPONSE IN DENTURE STOMATITIS

1. Introduction

The cellular immune response is thought to be of primary importance in combating systemic fungal infections[177]. The same would appear to be true of denture stomatitis, indeed the inflammation seen has been ascribed to a delayed-type hypersensitivity response[82,96,97]. In contrast it has been suggested that denture stomatitis is associated with suppression of the cellular immune response[178,179]. Most studies have used the inhibition of leucocyte migration as a measure of the cellular immune response in patients with denture stomatitis. In one study the phagocytosis of yeasts by leucocytes from patients with denture stomatitis was found to be normal[180]. In another study, no difference was found in the lymphocyte transformation response of patients with denture stomatitis and controls[181] although the controls do not appear to have been screened for candidal carriage and only one mitogen, phytohaemagglutinin (PHA), was used. If the cellular immune response is depressed in patients with denture stomatitis the possibility exists that this may be innate or induced by *Candida*. To try and clarify this, lymphocyte transformation tests were performed using patients with denture stomatitis, other oral candidosis and healthy controls who were either carriers or non-carriers of *Candida*. In addition, by freezing lymphocytes it was

possible to examine the lymphocyte transformation response in the same patient before and after antifungal treatment.

2. Materials and Methods

Approximately 30 mls of heparinised venous blood were obtained from patients with denture stomatitis, patients with candidal leukoplakia and controls who had been determined positive or negative for candidal carriage. Blood was obtained from two denture stomatitis patients and two leukoplakia patients both before and after antifungal treatment.

Twenty five ml of whole blood, previously diluted 1:2 with RMPI 1640 medium was layered onto 20 ml of a premixed commercial preparation of Ficoll-Hypaque (Lymphoprep, Flow Laboratories, Rickmansworth, UK) in 50 ml polystyrene disposable tubes. The tubes were then centrifuged at 20 °C for 25 minutes at 400xg. Mononuclear lymphocytes and monocytes were recovered at the Ficoll-Hypaque-plasma interface and washed twice in RMPI 1640. The leucocyte suspensions were then diluted with dimethyl sulphoxide (Sigma, Poole, Dorset, UK) and placed in a cryopreserving freezer and stored at -70 °C. Prior to performing the lymphocyte transformation assay the leucocyte suspensions from the appropriate subjects

were defrosted, washed three times with phosphate buffered saline and cell viability checked using the trypan blue dye exclusion test. The leucocytes were counted in an automatic cell counter and adjusted to an appropriate working concentration.

The leucocytes were cultured in round bottomed microtitre culture plates (Flow Laboratories, Rickmansworth, UK). The cultures, in triplicates, contained $2x10^5$ leucocytes in a final volume of 0.2 mls consisting of RMPI 1640 supplemented with 10% foetal calf serum, 100 units/ml of penicillin and 100 ug/ml of streptomycin.

Leucocyte proliferation was induced by PHA at a concentration of 1.2ug/ml, pokeweed mitogen at a concentration of 1ug/ml and heat-killed *C. albicans* at a concentration of $1x10^6$ organisms/ml. The cultures were incubated for three days for mitogens and five days for antigen at 37 °C in a humidified atmosphere of 5% carbon dioxide in air. ^{3}H-thymidine (Amersham International PLC, Amersham, UK) in a dose of 0.5 uCi per well, was added for the last four hours of incubation. A set of triplicate cultures containing no mitogen or antigen served as the control for background thymidine incorporation.

The cultures were harvested using a semi-automated cell harvester (Titertek, Flow Laboratories, Rickmansworth, UK) onto glass-fibre filter paper. The fibre-glass filters, containing the harvested cells, were immersed in vials containing 3 mls of scintillation fluid (Fisons Scientific Apparatus, Loughborough, UK) and the radioactivity counted in a liquid scintillation counter (Pakard Tri-Carb Ltd., Caversham, UK). The amount of ^{3}H-thymidine incorporated by the cells was expressed as the mean counts per minute (cpm).

The materials and methods described are essentially the same as those described by Wray[235], except for the freezing of the leucocytes. Six separate assays were performed, each containing leukocytes from one control positive for candidal carriage, one control negative for candidal carriage, one denture stomatitis patient and one candidal leukoplakia patient. For two of the six assays leukocytes were tested which had been obtained from the denture stomatitis patient prior to and after antifungal therapy. Also in two assays, leucocytes were tested from patients with candidal leukoplakia, pre and post treatment.

3. Results

Tables 47 to 52 give the results of the six assays, expressed in mean counts per minute for each set of leucocyte cultures. The stimulation index is given in parentheses beneath the relevant cpm figure. The stimulation index is calculated by dividing the mean cpm of the stimulated cells by the mean cpm of the unstimulated control cells. An explanation of the abbreviations used can be found at the end of the results section.

TABLE 47: LYMPHOCYTE TRANSFORMATION ASSAY 1.

PATIENT	PHA	PWM	C.ALB	CELLS
Control+	87,912	67,440	3547	42
	(2093)	(1606)	(84)	
Control-	16,557	71,450	22,780	129
	(128)	(554)	(176)	
DS	24,273	68,450	4759	40
	(607)	(1711)	(119)	
Leuk	71	70	95	35
	(2)	(2)	(2.7)	

TABLE 48: LYMPHOCYTE TRANSFORMATION ASSAY 2.

PATIENT	PHA	PWM	C.ALB	CELLS
Control+	41,422	97,797	103	42
	(986)	(2328)	(2.4)	
Control-	136,425	58,570	10,831	171
	(798)	(342)	(63)	
DS	189,057	82,919	25,407	66
	(2864)	(1256)	(385)	
Leuk		42,260	2820	66
		(640)	(43)	

TABLE 49: LYMPHOCYTE TRANSFORMATION ASSAY 3.

PATIENT	PHA	PWM	C.ALB	CELLS
Control+	32,150	16,911	21,124	230
	(140)	(73)	(91.8)	
Control-	47,585	42,508	1232	48
	(991)	(885)	(25.6)	
DS pre	51,458	72,783	500	49
	(1050)	(1485)	(10.2)	
DS post	59,444	21,843	2694	57
	(1043)	(383)	(47.3)	
Leuk	67,255	58,826	11,911	167
	(403)	(352)	(71)	

TABLE 50: LYMPHOCYTE TRANSFORMATION ASSAY 4.

PATIENT	PHA	PWM	C.ALB	CELLS
Control+	65,085	68,016	15,591	63
	(1033)	(1080)	(247)	
Control-	32,112	52,827	14,143	105
	(306)	(503)	(135)	
DS pre	20,791	68,528	1972	28
	(742)	(2447)	(70)	
DS post	31,395	68,880	11,091	39
	(805)	(2535)	(284)	
Leuk	86,412	56,619	20,404	1457
	(59)	(39)	(14)	

TABLE 51: LYMPHOCYTE TRANSFORMATION ASSAY 5.

PATIENT	PHA	PWM	C.ALB	CELLS
Control+	48,904	87,050	2495	97
	(504)	(897)	(26)	
Control-	78,008	71,840	12,807	50
	(1560)	(1437)	(256)	
DS	30,669	83,580	1964	166
	(185)	(503)	(12)	
Leuk pre	42,118	48,275	8762	104
	(405)	(464)	(84)	
Leuk post	53,321	59,697	25,291	99
	(538)	(603)	(255)	

TABLE 52: LYMPHOCYTE TRANSFORMATION ASSAY 6.

PATIENT	PHA	PWM	C.ALB	CELLS
Control+	110,031	86,859	14,828	487
	(226)	(178)	(30)	
Control-	161,321	84,595	12,521	62
	(2601)	(1364)	(202)	
DS	123,958	442	20,824	42
	(2951)	(10)	(496)	
Leuk pre	96,200	72,436	912	62
	(2237)	(1684)	(21)	
Leuk post	79,714	76,301	3978	35
	(2277)	(2180)	(114)	

Abbreviations:

PHA, Phytohaemagglutinin

PWM, Pokeweed mitogen

C.ALB, *Candida albicans*

CELLS, Unstimulated control cells

Control+, Control patient positive for candidal carriage

Control-, Control patient negative for candidal carriage

DS, Denture stomatitis patient

Leuk, Candidal leukoplakia patient

pre, before antifungal treatment

post, after antifungal treatment

4. Summary of Results and Discussion

There was no consistent relationship between the lymphocyte response of control patients who were or were not carriers of *Candida*. However in four of the six assays the stimulation index of carrier leucocytes challenged with *C. albicans* was less than that of non-carrier leucocytes. If *Candida* were responsible for causing depression of cellular immune response a graded lymphocyte response to *C. albicans* might be anticipated, with the highest stimulation index in non-carriers, the next highest in healthy carriers and the lowest in patients with clinical candidal infection. Such a gradation could not be consistently demonstrated.

In four of the six assays leucocytes from patients with candidal leukoplakia showed a lower stimulation index than those from patients with denture stomatitis when challenged with *C. albicans*. The same relationship was generally true for mitogen stimulation also.

The most interesting feature of the results relates to the effect of antifungal treatment. Antifungal treatment of both denture stomatitis and candidal leukoplakia always led to an increased stimulation index in leucocytes callenged with *C. albicans*, compared with that seen in leucocytes from the same patients before

treatment. The response of leucocytes to stimulation with mitogens remained unchanged by antifungal treatment. This would seem to suggest that Candidal infection can cause depression of the cellular immune response but that this may be confined to the ability to respond to *Candida* antigens. The cellular immune response to *Candida* antigen would seem to be restored following antifungal therapy. Expansion of this preliminary study was not possible owing to the limitations of time and funding. The small numbers involved make statistical analysis inappropriate. The trends seen, however, are interesting and further studies are warranted.

CHAPTER SEVEN

DISCUSSION AND CONCLUSIONS

The use of antifungal drugs is an acceptable option for the treatment of denture stomatitis[62]. The rationale for such treatment is based on compelling evidence indicating that yeasts are the prime cause of the disease (see Chapter 1). Indeed the improvement seen during use of antifungals is often cited as proof of the causitive role of yeasts in denture stomatitis. However antifungal drugs are frequently only partially successful in alleviating the signs and symptoms of the disease[15,69,182,185,186]. Furthermore, once treatment is stopped, relapse and recurrence are very common[12,29,32,69,183]. Explanations for the often disappointing outcome of antifungal therapy have included re-infection from exogenous as well as endogenous sources[15,17,182], in addition to the involvement of other factors in the aetiology of the condition[18,185]. Despite the fact that the aetiology of denture stomatitis is said to be multifactorial[61,62] the influence of these other factors on the efficacy of antifungal therapy has rarely, if ever, been properly investigated. The broad aim of the work reported in this thesis was to conduct just such an investigation.

Inquiry into the effect of a number of factors on the outcome of antifungal therapy might help to shed light on the inter-relationship of these factors in the aetiology

of denture stomatitis. In this respect the term "multifactorial" requires some consideration. It could be taken to mean two things: firstly that a number of factors could act totally independently to produce conditions with very similar signs and symptoms, and secondly, that a number of factors may act together to produce the signs and symptoms of the condition. In the first case there would be a number of conditions which, whilst clinically indistinguishable, would have quite separate causes, and in the second case one condition might have a varying number of factors contributing to the aetiology. The literature is not helpful in deciding which of these definitions is most appropriate in describing the aetiology of denture stomatitis.

A previously published trial had indicated that fluconazole was an effective treatment of denture stomatitis when compared with a placebo[13]. The next logical step was therefore a comparative trial of this new drug with a drug such as amphotericin, which, although useful, had demonstrated some of the shortcomings in efficacy described above. Not only would two different types of antifungal agent be compared but two different modes of administration, which, if theories about re-infection from endogenous sites and patient compliance were correct, might significantly effect the outcome of treatment.

Fifty nine patients were entered into a comparative trial of the two antifungal agents. The patients were randomly assigned to recieve either fluconazole or amphotericin, the first of these being a systemically administered and the second a topically administered antifungal drug. A number of variables which might possibly have influenced the outcome of treatment were recorded and where possible quantified. These variables included the initial severity of denture stomatitis, the patients' sex and smoking status, denture wearing habits, the type and quality of the patients' denture, haematologic abnormalities, the yeast species involved and the patients compliance with treatment. In terms of these variables the randomisation process produced quite evenly matched treatment groups. There were some slight differences: on average, patients in the amphotericin group were slightly older (60 years) than those in the fluconazole group (50 years), the fluconazole group had a slightly higher percentage of smokers than did the amphotericin group and whilst the amphotericin group had slighlty more complete upper than partial upper denture wearers, complete and partial upper dentures were equally distributed in the fluconazole group. For all the other variables, observed differences between treatment groups, where they occurred, were only minor.

Fifty nine patients might be thought a small number. As far as the known variables are concerned 59 proved to be a sufficient number to allow the randomisation process to work effectively. It seems reasonable to assume therefore, that unknown variables were also evenly distributed between the treatment groups. The size of an experimental group also determines the ability of a study to detect real differences where they occur. Clearly the larger the sample the better. The comparisons reported in this thesis did demonstrate statistically significant differences in some cases. In fact 59 is a greater number of patients than have been involved in most previously reported studies of antifungal treatment of denture stomatitis[10,12,13,182,185,188-190,212], all of which have involved fewer investigations than the present study.

Before considering the clinical and mycologic efficacy of the drugs some of the presenting features of the patients will be discussed. There were almost two and a half times as many women as men. The vast majority of these patients were otherwise in good health and the incidence of haematologic deficiency was small (five patients had a low serum ferritin and two had a low serum folate). Just over half the patients wore a complete upper denture, the remainder wearing a partial upper denture. Only one third of these dentures were judged to be of a good overall

quality. In particular, denture retention, stability and occlusion were often poor. The commonest type of denture stomatitis was diffuse erythematous, accounting for slightly more than one half of the patients. The remaining patients had localised erythematous or hyperplastic/granular forms of the condition in approximately equal numbers. Symptoms were reported by 52.5% of patients, the most frequent complaint being of dry mouth. Symptoms occurred most commonly in those with the hyperplastic/granular from of denture stomatitis. The commonest concomitant oral candidosis was angular cheilits which occurred in 18.6% of patients and which was bilateral in each case.

The proportion of patients with the diffuse form of denture stomatitis was higher than in previous studies[46,50,51,54,55,59] whilst fewer patients had angular cheilits than might have been expected[8,15,18,25,27,33-35]. The proportion of patients with symptoms was similar to that previously reported[27,29]. However the majority of symptoms were mild and general awareness of the condition was low. Comparison of these presenting features with those reported in previous studies may be misleading. In this study the patient sample was highly selected. Potential patients were frequently excluded from entry into the study based on the criteria described in chapter two.

The small number of haematologic deficiencies were not corrected and this did not appear to influence the outcome of treatment in these patients. This lends support to previous studies which have been unable to demonstrate a relationship between iron and folate deficiencies and denture stomatitis[150,151].

Maximum clinical efficacy was observed for both drugs at the four week visit, when 84% of fluconazole and 91% of amphotericin patients were cured or improved. At this stage it is likely that the number of cured and improved patients in the fluconazole group was already declining since they had completed treatment two weeks previously, in accordance with the protocol. The design of the trial, therefore, probably did not allow the maximum clinical efficacy of fluconazole to be detected. However this would appear to be unimportant given the high rate of relapse and recurrence observed by the 12 week follow-up visit.

The difference between treatments, in terms of clinical response, was not significant at any visit. Fluconazole treatment was completed after two weeks and amphotericin treatment after four, and yet at the four week visit there was no significant difference between the two treatment groups. This may indicate that the improvement

produced by fluconazole was more prolonged. The trend seen at the 12 week visit, when 58% of fluconazole patients were cured or improved compared with 50% of amphotericin patients, may support this assumption. If this were the case then a possible explanation could be the systemic effect of fluconazole, which might reduce the chances of re-inoculation of yeasts from endogenous sites such as the vagina or the gut. However this study does not provide convincing evidence to support this theory.

Budtz-Jorgensen et al[13] reported 16% of patients with denture stomatitis were cured and 73% improved after two weeks of fluconazole treatment. The total of 89% cured or improved by fluconazole compares well with the 84% cured or improved seen in the present study two weeks after the completion of treatment. However, in the present study 44% of patients were cured. A similar means of patient selection was used in both studies and although different means of assessing improvment in palatal erythema were used, "cure" would seem to be unequivocal. In the study by Budtz-Jorgensen et al[13] patients were given no instructions regarding denture hygiene, whereas in the present study they were. Although compliance with such instructions is difficult to estimate this does seem to be a likely explanation for the difference in cure rates between the two studies. Furthermore in the

Budtz-Jorgensen et al study[13] relapse had occurred in virtually all patients by four weeks after the completion of treatment. In the present study, although there was considerable relapse and recurrence, at the twelve week visit, at least half the patients in both treatment groups were still cured or improved. Good denture hygiene may have contributed to the sustained improvement seen in these patients. It therefore seems reasonable to suggest that a combination of denture hygiene measures and antifungal treatment results in a more pronounced and prolonged improvement in denture stomatitis than antifungal treatment alone.

It is interesting to note that patients with each classification of denture stomatitis responded equally well to antifungal treatment. This contradicts the view that the localised form of the condition is caused by denture trauma and suggests instead, that it is a mild form of *Candida*-associated denture stomatitis.

The symptoms of pain, burning sensations, dry mouth and abnormal taste were cured or improved by antifungal treatment in the majority of patients and there were no significant differences between fluconazole and amphotericin. It is not possible to say with certainty why antifungal treatment failed to improve some patients' symptoms. This may have been due to poor drug efficacy

but it may also have been that the symptoms had other causes. Where symptoms were improved it seems likely that they were associated with denture stomatitis. However a placebo effect cannot be discounted. It is interesting that over half the patients who complained of dry mouth reported this symptom to be resolved or reduced following antifungal treatment. It seems likely that a feeling of dryness of the mouth may, in some cases, be caused by oral candidosis and this may have contributed to the belief that xerostomia predisposes to such infections.

Antifungal treatment also resulted in the improvement of a number of concomitant oral conditions. In the case of angular cheilitis this may appear to implicate yeasts in the aetiology. However improved denture plaque control may also have reduced the bacterial population in the mouth. Previous studies have indicated that the infective aetiology of angular cheilitis is complex and that in many cases bacterial infection is more significant than candidal infection[38,39].

Fuller explanation of the clinical response requires consideration of the mycologic events. Mycologic cure was taken to be complete eradication of yeasts from the mouth (i.e. when no yeasts were cultivated from any of the six sites sampled by swabbing or from the oral

rinse). In patients not rendered yeast free a decrease in the density of yeast growth was taken to indicate improvement. This approach has been adopted in other studies[10,13,64,69,182,185,186,190]. However the methods used in many of these studies could only be described as semi-quantitative. Methods for quantitative assessment of yeast colonisation of oral surfaces have been compared[228] and the imprint culture and concentrated rinse culture methods found to be equally sensitive. The concentrated rinse method was found to be superior for quantifying very high densities of organisms. An oral rinse culture was used in the present study but two problems were encountered. Firstly, there are no data available to suggest whether denture wearers should have their dentures in or out of the mouth during rinsing. If the dentures are out of the mouth then the denture fitting surface, which is a prime site of yeast growth, will not be sampled. However if the dentures are worn during rinsing it is still possible that the denture fitting surface will not be sampled and in addition the covered area of the palate may not be sampled. Most patients in the present study rinsed with their dentures out but some found it difficult to expectorate unless the dentures were worn. These patients wore their dentures when rinsing. Hence comparisons of yeast density between patients cannot be made. However patients either wore or did not wear their dentures consistently throughout the

study and it is therefore possible to make comparisons between visits for individual patients.

A very wide range of colony counts was obtained and it was not possible to identify figures representative of infection or improvement. Indeed it may be quite impossible to identify such figures since it seems likely that host response to differing levels of candidal loading would vary between individuals. This led to the second difficulty with the oral rinse technique, namely determining what level of reduction in colony count, in comparison with the baseline assessment, was representative of improvement. An entirely arbitrary level of 50% or greater reduction in colony count was set as the criterion to indicate that a mycologic improvement had occurred in an individual patient.

The best mycologic response was seen at one week in both treatment groups. After this assessment the number of patients mycologically cured or improved gradually declined, representing re-colonisation in patients who had been yeast free, and a return to pre-treatment yeast densities in others. No more than half the patients were mycologically cured at any time. At one and four weeks the number of mycologic cures was greater in the amphotericin group than in the fluconazole group and at four weeks this difference was statistcally signifcant.

The difference at four weeks is not surprising given that the amphotericin group received a longer course of treatment. What is perhaps surprising is that at four weeks the mycologic response in the amphotericin group had already begun to decline when most patients had only very recently completed treatment.

Apart from the length of treatment, there are other possible explanations for the slightly better mycologic response seen in the amphotericin group. In order to be effective an antifungal drug must reach the site of infection and yeast proliferation. In denture stomatitis it must reach the palatal mucosa and the fitting surface of the upper denture. This is dependent both upon patient compliance and the mode of drug adminstration. Compliance with treatment is difficult to assess but in the present study only two patients were excluded from efficacy analyses owing to admitted poor compliance. Both patients were in the amphotericin group. The commonest reported side effect of treatment was nausea. Three patients reported this problem and all were in the amphotericin group. Subjectively it seems reasonable to expect patients to comply more readily with taking one capsule daily for two weeks than with sucking four lozenges daily and spreading cream on the fitting surface of the denture for a month. Amphotericin lozenges were allowed to dissolve in the mouth with the dentures removed.

Amphotericin cream was then applied directly to the fitting surface of the denture. Assuming good compliance, therefore, amphotericin would have had good access to yeasts in the denture plaque and on the palatal mucosa. Fluconazole is well distributed in saliva and serum. The drug may therefore have had access to the palatal mucosa and denture plaque via serum exuded through the palatal lesions. However the extent to which this would have occurred is unknown. If saliva was unable to flow freely beneath the fitting surface of a retentive denture the effectiveness of fluconazole would be reduced. Provided the patient removed the dentures at night, fluconazole in saliva would have some access to the palatal mucosa. However it is conceivable that, in some cases, the drug had no access to the denture plaque at all.

A further interesting difference between fluconazole and amphotericin was observed, that was in regard to the effect on yeast species. *Candida albicans* was isolated from approximately 70% of sites at the baseline assessment and was by far the commonest species identified. Both fluconazole and amphotericin were effective in reducing the frequency of isolation of this species. *Candida glabrata* was isolated in 11 patients at the baseline assessment (16.4% of all sites). Amphotericin was effective in reducing the frequency of isolation of *C. glabrata* but in the fluconazole group

the frequency of isolation of this species remained the same throughout the study, representing the persistent isolation of the organism from the same patients. This suggests that the strains of *C. glabrata* present were resistant to fluconazole. This finding confirms two previous reports of resistance of *Candida glabrata* to fluconazole, even when administered in high doses[237,238]. The numbers in the present study were too small to prove an association between the persistence of *C. glabrata* and clinical failure of treatment. However infection with *Candida glabrata* should be suspected in patients with denture stomatitis or any other form of oral candidosis, who fail to respond to fluconazole treatment. Ideally yeasts should be cultivated and the species identified before treatment is commenced but this does not rule out the possible emergence of resistant organisms at a later stage.

Having considered the mycologic changes brought about by antifungal treatment it is necessary to inquire how far these changes explain the clinical response observed. In general terms the mycologic and clinical responses were similar. Treatment brought about an improvement which was followed by relapse. In terms of the whole patient group, clinical response appeared to lag behind mycologic response. This seems reasonable; elimination of infection is followed by healing of the lesions and re-infection is

followed by a return of signs and symptoms. However when individual patients were studied the relationship between clinical and mycologic responses was far from clear.

The clinical and mycologic responses for each individual patient at each follow-up visit were examined to see how often the two "agreed". It was taken that failure to show a mycologic improvement would be accompanied in the same patient by failure to improve clinically. Similarly mycologic improvement should be accompanied by clinical improvement. At the four week visit such agreement was found in only 30% of fluconazole patients and 60% of amphotericin patients. If there was a true time lag between clinical and mycologic response it might be more appropriate to compare the mycologic response at one visit with the clinical response at the next visit. When this theory was examined correlation was only marginally better. This poor correlation prompted examination of other possible indicators of mycologic improvement. The palate, fitting surface of the denture and the tongue were the commonest sites of yeast isolation. By the one week visit yeasts had been eliminated from approximately half of these sites. Failure to cultivate yeasts from the palate and/or the fitting surface of the denture might be considered to be a reasonable indicator of mycologic improvement. When tested against clinical response, this mycologic indicator did indeed show better correlation.

At the four week visit there was agreement between mycologic and clinical response in 52% of fluconazole patients and 73% of amphotericin patients. As an indicator of mycologic improvement, therefore, failure to grow yeasts from the palate or denture seems more relevant to the clinical response than does reduction in oral rinse derived colony counts.

Nonetheless, using either mycologic indicator, correlation between mycologic and clinical events was less than might be expected for a disease considered to be primarily a yeast infection. An explanation for this might be the inherent imprecision in both clinical and mycologic assessments. The difficulties of assessing clinical improvement have been discussed (see Comment on Clinical Methods, Chapter 3). The elimination of subjectivity and ensuring consistency of judgement over the course of a study will present problems whatever method is used. In at least one other study, palatal erythema was scored and lesions photographed and correlation between mycologic and clinical events was still found to be poor[13]. There appears to be no guarantee that increasing the apparent sophistication of an assessment also increases its reliability. Indeed improvement judged, for example, by a change of one point on a five point scale, may be clinically irrelevant. The merit of the method adopted in the present investigation

was that "no change, marked improvement or cure" at least has relevance to the everyday clinical situation.

The limitations of the oral rinse technique for the quantitation of yeasts in the mouths of denture wearers have already been discussed. In addition the use of a 50% reduction in the resulting colony counts may have been inappropriate as an indicator of improvement. However there is no suggestion in the literature of what might be considered a significant reduction. Failure to grow yeasts from swabs of the palate and denture might be thought less equivocal. In fact the growth of yeasts from swabs may rely upon such factors as the area swabbed, the pressure used and the means of transport to the laboratory. However it does not seem unreasonable to suggest that elimination of yeasts, as judged by cultivation from swabs, is indicative of a drastic reduction in their number.

It is clear that comparison of clinical and mycologic responses is, in effect, comparison of two estimates. It is therefore not surprising that they frequently fail to agree. However in the present study, when clinical response was unequivocal, correlation with mycologic events was clear. Patients who demonstrated an excellent clinical response, that is they were cured and remained cured throughout the study, were either mycologically

cured or showed a drastic, sustained reduction in the number of sites from which yeasts were isolated and in colony counts. Patients who remained clinically unchanged throughout the study showed continued heavy growth of yeasts at all sites. Hence when neither clinical nor mycologic events were open to doubt the correlation between the two was apparent.

From these observations it appears that sustained clinical cure of denture stomatitis is associated with elimination or near elimination of yeasts from the mouth. In those patients not cured but clinically improved, an association with reduction in yeast numbers was more difficult to demonstrate because of the imprecision involved in assessing both clinical and mycologic status. It is therefore necessary to look beyond individuals to the experimental group as a whole. Here the overall results suggest that clinical improvement is associated with mycologic improvement and that relapse and recurrence are associated with recrudescence of yeast infection.

The greatest number of patients deemed to be mycologically cured was observed at the one week visit but by the twelve week assessment the number of mycologic cures was considerably reduced. This raises a question as to the source of re-infection. One possible source is

yeasts from distant endogenous sites. Fluconazole performed slightly better than amphotericin with regard to long term mycologic cure. The systemic drug may have eliminated yeasts at other host sites thus reducing the chances of auto-inoculation of the mouth. It should be stressed again that if such an effect had occurred its results were not obvious. It seems more likely that yeasts were never truly eliminated from the mouth in a large majority of patients, even in those thought to be mycologically "cured". This is certainly possible, if antifungals have a limited effect on non-dividing organisms then a small number of yeast organisms could persist on oral and denture surfaces and not be detected by swabbing. Following completion of treatment, these organisms could be the source of re-population. In those patients with sustained improvement it is possible that changes in the oral environment, specifically, improved denture hygiene, could prevent this re-population occurring. Unfortunately, although patients were given instruction in denture hygiene, no assessment of this important factor was performed after the baseline visit. It is therefore not possible to say how denture hygiene changed during the study and this is a valid criticism of the experimental design. However it is revealing to look again at the very similar study of Budtz-Jorgensen et al[13], where patients were given no instruction in denture hygiene and where the mycologic response was

considerably worse than in the present study. The denture hygiene instruction given to patients in the present study would therefore appear to have been beneficial. However conclusions in this regard must remain tentative.

One further point concerning denture hygiene should be noted. Patients who were effectively cleaning their dentures were removing denture plaque which contains both yeasts and bacteria. The effect of reducing the bacterial population cannot be discounted. However denture hygiene will have varied in its effectiveness across the experimental group. Also, if plaque removal alone had been responsible for producing clinical improvement, a more constant level of improvement throughout the study might have been anticipated. Instead, at the four week visit, cure or improvement was almost universal; but by the twelve week visit there had been marked relapse and recurrence. Thus the timing of clinical improvement was closely related to that of antifungal drug use and relapse and recurrence was seen after drug use was discontinued. This suggests that the antifungal effect was of major importance in producing the clinical responses seen. The results therefore provide further evidence to implicate yeasts as the primary causative agent in denture stomatitis.

Compliance with the treatment regimen and with denture hygiene measures may explain why some patients responded better than others. However it has been demonstrated that the relationship between clinical and mycologic events was complex. It is possible that other variables may have influenced the outcome of treatment.

The clinical response in women was consistently better than that in men and this difference was significant at the one week assessment. Again better compliance is a possible explanation. The hormonal changes which occur during menstruation and pregnancy may cause exaggerated gingival inflammation in the presence of microbial plaque and the oral contraceptive pill may have similar effects[239]. Pregnant and lactating women were excluded from this study, indeed many of the women were probably post-menopausal, although hormone replacement therapy may also affect gingival inflammation[239]. However, if such hormonal states are sometimes responsible for increased inflammation in denture stomatitis, as seems plausible, a worse response to antifungal therapy, compared with that in men, might be expected. A possible explanation for the better clinical response seen in women arose when the effects of smoking were considered.

Patients who smoked tobacco showed a poorer clinical response than those who did not. This is the first study

to have demonstrated such a relationship. The difference between smokers and non-smokers was statistically significant at one week but by four weeks the clinical response in smokers appeared to have caught-up and the difference remained insignificant at the twelve week assessment. The response in ex-smokers tended to be intermediate between smokers and non-smokers, although there was only a small number of ex-smokers. Thus smoking appeared to slow down the clinical response. With regard to the difference in clinical response between the sexes it is revealing to note that 56% of women but only 19% of men were non-smokers.

Previous reports make almost no mention of a link between denture stomatitis and smoking. There is some epidemiological data to suggest that smoking is associated with an increased prevelance of denture stomatitis[47,54] but there is conflicting evidence concerning the effect of smoking on oral candidal carriage[144,145]. One recent study suggested that smoking was associated with a less homogenous oral mycoflora when compared with non-smokers and that this may be an important factor in oral candidosis[240].

The effect of smoking on periodontal disease is well documented. Smoking would appear to be a major risk factor for periodontal disease[241]. Whereas it was

previously thought that the association between periodontitis and smoking was due to indirect effects, such as poorer oral hygiene in smokers, it now appears as though tobacco smoke exerts direct effects both locally and sytemically[241]. Smoking would appear to have an adverse effect on the outcome of periodontal treatment[242,243]. This does not seem to be caused by differences in the occurrence of periodontal pathogens in smokers and non-smokers[244], but may well be related to the effects of smoking on the host response[245,246].

In the present study smoking appeared to give rise to differences in the initial presentation of denture stomatitis. The hyperplastic/granular form of the disease was far commoner amongst smokers than amongst non-smokers. It is not certain that this is a causal relationship and if it were, the mechanisms behind it seem obscure. However the difference may have influenced antifungal drug efficacy since it may have been difficult to eliminate yeast organisms from the deep epithelial crypts in the hyperplastic/granular form of denture stomatitis. In fact the results do not support this view since the clinical response was similar in patients with each type of denture stomatitis.

Refractory periodontitis refers to periodontal disease which has persistently failed to respond to conventional therapy. In the present study of 59 patients with denture stomatitis, antifungal treatment failed to produce any improvement in six patients. Of these six patients, four were smokers. In contrast eight patients were cured and remained cured throughout the study. Of these eight only two were smokers. Although the numbers of very poor and excellent responders are small there is a suggestion that "refractory" denture stomatitis may be associated with smoking.

It seems unlikely that smoking would exert a local effect in denture stomatitis since the area concerned is usually covered by the denture. However a systemic effect is possible and this will be considered in more detail when the immune response is discussed.

The influence of prosthodontic factors on the clinical outcome of antifungal therapy was examined. Patients who removed their dentures at night were no more or less likely to be cured or improved than those who wore their dentures constantly. Of course this judgement is dependent upon accepting the truthfulness of the patients' replies when questioned on this matter. Previous studies have commented on denture wearing habits largely in relation to the prevelance and aetiology of

denture stomatitis. Whilst some studies claim to have shown a relationship between constant denture wearing and denture stomatitis[22,27,34,42,55], others have failed to do so[25,35,137]. However, these studies have largely failed to consider the relationship between denture plaque levels and constant denture wearing. Advice given to patients to remove their dentures at night is presumably based on the view that this will lessen the time during which the mucosa will be subjected to insult either through trauma or from the micro-organisms in denture plaque. If the infective aetiology of denture stomatitis is accepted it would seem irrelevant whether a patient with a perfectly clean denture removes it or wears it at night. On the other hand, in a patient with moderate or poor denture hygiene, constant wearing of the denture may lead to more severe inflammation of the denture bearing mucosa. Support for this view may be derived from one study in which severe inflammation was found to be commoner in constant denture wearers, whilst more moderate inflammation occurred as frequently in those who did not wear their dentures at night as in those who did[42]. With regard to treatment, therefore, it may be that once yeast levels have fallen to a certain level, the difference between constant and day-time only denture wearers becomes unimportant.

Constant denture wearers taking amphotericin did not show a better clinical response than those taking fluconazole. The potential for continual topical drug application therefore appears to have conveyed no particular benefit.

Clinical response was slightly better in complete denture wearers than in partial denture wearers, although the difference was not significant at any visit. Oral hygiene may be more difficult in partially dentate patients and supra and subgingival dental plaque may harbour organisms which serve to constantly repopulate mucosal and denture surfaces.

Overall, denture quality factors did not appear to be important in determining the clinical outcome of antifungal therapy. The clinical methods used for assessing dentures were, by nature, imprecise. No method of clinical judgement of denture quality can be without drawbacks. However all assessments were made by the same clinician who was unaware of the treatment prescribed. The judgements should therefore have been reasonably consistent and there is no reason to assume bias. A significant association between denture quality and clinical outcome was demonstrated for two of the six factors assessed, but only at the twelve week visit. At this time patients with "fair" denture retention showed a better clinical response than those with "good" or

"poor" retention. Also at twelve weeks patients with "poor" denture occlusion showed a better clinical response than those with "fair" occlusion, who in turn did better than those with "good" occlusion. It is difficult to explain these findings but given the overall results relating to denture quality, their importance should not be over emphasised. No prosthodontic treatment was provided for any patient during this study and at the four week assessment most patients were clinically cured or improved. In addition, for each denture quality factor assessed, patients with "good", "fair" or "poor" dentures responded equally well. These results strongly suggest that denture quality and, by implication, trauma from dentures, are not primary factors in the aetiology of denture stomatitis.

Proponents of denture trauma as a major cause of denture stomatitis have never satisfactorily explained why denture stomatitis is so rarely seen in the mandibular denture bearing mucosa. Prosthodontists know that a lower complete denture is likely to be more traumatic than an upper complete denture since it is usually less stable and it has a much smaller denture bearing area to resist occlusal forces. However it is possible that dentures may be traumatic in another sense, if they are constantly and closely applied to the mucosa. The denture fitting surface, covered with plaque, will be held firmly against

the mucosal surface. The toxins and antigenic components within that plaque are almost bound to elicit a response in the tissues. These toxins and antigens would be undiluted by saliva, the flow of which, beneath a well fitting denture, would be restricted. This would apply especially to the upper denture where good retention can often be achieved, in contrast to that which can be obtained in the lower. In the absence of the mechanical washing effect of saliva and of its antimicrobial content, an infection could become established. This would explain why denture stomatitis is seen almost exclusively in the maxilla. Denture trauma in this sense requires the presence of denture plaque.

If trauma from dentures is not a primary causative factor in denture stomatitis, it may nonetheless be a contributory factor and there are two potential models for the relationship between trauma and infection. Firstly denture trauma, however defined, may be a predisposing factor. It is axiomatic that denture stomatitis cannot occur in the absence of a denture so that in a sense, the mere presence of a denture predisposes to denture stomatitis. However trauma from a denture is conventionally understood to mean something more than its simple presence in the mouth. One way in which trauma may predispose to denture stomatitis has been described in the preceding paragraph. Another is

that mechanical trauma may give rise to inflammatory exudate which could enhance the adhesion of yeast organisms to acrylic[112]. The second model to explain the relationship between denture trauma and infection is that mechanical trauma could result in enhancement of an inflammatory response initiated by yeast infection.

Whether or not trauma from dentures is a predisposing or exaggerating factor, it is clear that if the infective component is removed the condition should resolve. The results of the present study support this view. If trauma from dentures predisposes to denture stomatitis it might be expected that patients with potentially traumatic dentures would be more prone to recurrence. At the twelve week follow-up, patients with "fair" denture retention and "poor" denture occlusion seemed least likely to suffer recurrence and this, together with the insignificant effect of any other denture quality factor, would appear to contradict the view that mechanical trauma predisposed patients to recurrence of denture stomatitis following treatment.

One of the denture quality factors assessed was denture hygiene. This study employed a method of assessing denture hygiene similar to that used in previous investigations[46,51,231]. However the use of a plaque disclosing solution would probably have produced more

useful results. The role of denture hygiene in the clinical responses seen in this study has already been discussed and its benefit deduced by comparison with other reports.

At the baseline assessment the denture hygiene of non-smokers tended to be slightly better than that of smokers. However given the limitations of the method of assessment used this finding should be viewed with caution. In any case the initial denture hygiene assessment did not appear to be related to clinical outcome and would therefore not explain the poorer outcome seen in smokers.

Total and specific anti-candidal antibodies were measured in serum and saliva throughout the study. Antibody levels were found to vary widely between patients and only very small changes were observed between visits. The absence of a marked effect of antifungal treatment on antibody levels is in agreement with some previous reports[169,178] but in disagreement with others[171,173]. None of these previous studies involved measurement of both serum and salivary antibodies. The wide range of antibody levels observed in the present study is probably indicative of considerable individual variation in the immune response to yeast organisms. The failure of antifungal agents to bring about a reduction

in antibody levels is probably related to the kinetics of the immune response. It is likely that yeasts were completely eliminated in only a very few patients and even in these re-infection occurred quite rapidly. It is perhaps not surprising, therefore, that the humoral immune response appeared to be sustained throughout the twelve week study period, particularly since in most patients the infection was of a long standing nature.

It was difficult to relate the humoral immune response to the density of yeast colonisation in the mouth. This may be partially explained by the method used for quantitation of yeast organisms, the possible limitations of which have already been mentioned. In addition yeast colonisation at other host sites would also presumably stimulate an immune response which would effect serum antibody levels. Colonisation of the gut would also effect the mucosal immune system and possibly, therefore, antibody levels in saliva. The influence of such factors in the present study is unknown but it is small wonder that the immune response varied so much and that it was difficult to relate both to oral yeast levels and to clinical outcome. Even if the level of yeast colonisation throughout the body had been the same for each individual it is likely that the immune response would still have varied.

One factor which was seen to exert a significant effect on the humoral immune response was smoking. Immunoglobulin levels, especially in serum, were frequently significantly higher in non-smokers than in smokers. In particular total serum IgG, total serum IgA, and serum anti-candidal IgG were effected. This would appear to be the first report of such an effect in patients with denture stomatitis and indeed in oral candidosis in general. This may help to explain why the clinical outcome of antifungal therapy in smokers was poorer than in non-smokers.

It is now well established that tobacco smoking may have wide ranging effects on the immune system[247]. With possible relevance to oral disease, smoking has been shown to cause depression of salivary IgA[248,249] although these studies did not involve patients with oral candidosis. Smoking has also been shown to impair serum IgG levels against specific periodontal pathogens[246]. However understanding of the effects of smoking on the immune response remains incomplete and relating the specific immune defects seen to clinical disease presents considerable difficulty[250].

The effects of smoking on periodontal disease are believed to be reversible[241] and the present study suggests that this may also be true for the effects on denture stomatitis since ex-smokers tended to show a clinical response intermediate to that of smokers and non-smokers.

The cellular immune response was studied seperately in a preliminary study employing a lymphocyte transformation test. A consistent finding in two patients with denture stomatitis and two patients with candidal leukoplakia was an increased stimulation index of leukocytes challenged with *Candida* antigen after antifungal treatment compared with that seen in leucocytes obtained before treatment. When challenged with PHA and pokeweed mitogen however, the stimulation indices of leucocytes obtained from these patients before and after treatment were similar. This may suggest that chronic candidal infection causes suppression of the cellular immune response but that this effect is quite specific. It may be that only the ability to respond to *Candida* antigen is inhibited, although other antigens were not tested. There has been only one other study of lymphocyte transformation in denture stomatitis and in this only PHA mitogen was used[181]. No difference was found between the response of control and patient leucocytes and the study is, in this respect, in agreement with the present study. In one

study lymphocytes from patients with unspecified chronic candidosis had a lower stimulation index than controls when challenged with *Candida* antigen but the PHA induced lymphocyte transformation was similar in both groups[174]. These findings correlate well with those of the present study. Studies using the leucocyte migration test have also suggested that cellular immunity is depressed in patients with denture stomatitis[178]. Suppressor T cells have been shown to emerge in cultures of human lymphocytes stimulated by a polysaccharide from *Candida albicans*[251] and this mechanism could be involved in the depressed cellular immunity seen in denture stomatitis.

The effects of smoking on the cellular immune response were not examined in the present investigation. However smoking is known to effect cellular immune function[247], including migration and phagocytosis in polymorphonuclear leucocytes derived from the oral cavity[252,253].

In an experimental model in monkeys, the cellular immune response was found to develop concomitantly with the clearing of a denture stomatitis-like infection which had been induced by inoculation of yeasts beneath an acrylic plate[96]. It is important to note that the infection resolved spontaneously, possibly as a result of the death

of the yeast organisms. It may be, therefore, that cellular immunity developed as the suppressive effect of *Candida* decreased. The humoral immune response developed later and as the antibody titre rose the cellular immune response declined. In animals treated with azathioprine the infection persisted although the clinical picture was changed from one of erythema to one of thrush. In these animals the cellular immune response was suppressed but the humoral immune response was strong and early. This would seem to indicate that cellular immunity is associated with clearing of the infection in this model and that when this is suppressed humoral immunity develops rapidly as a second line of defence. However this model has only limited relevance to denture stomatitis in humans which is often chronic in nature and in which yeasts would appear to flourish in a favourable environment. The depression of cellular immunity in denture stomatitis would appear to be due to chronic candidal infection and it would not be surprising if its effects were less dramatic than those produced by drugs such as azathioprine.

The association between humoral and cellular immune responses in humans with denture stomatitis has been studied and an inverse relationship between the two demonstrated[178]. Cellular immunity was restored following antifungal therapy but serum antibody levels

remained the same. Cellular immunity was shown to be particularly depressed in patients with the hyperplastic/granular form of denture stomatitis.

From the results of the present investigations and those of previous studies a possible model for the immune response in denture stomatitis can be postulated. Chronic candidal infection causes a degree of suppression of the cellular immune response. The humoral immune response, though present, is overwhelmed by yeast organisms which are continually being replenished. The humoral immune response is therefore unable to clear the infection but is able to confine it. Serum antibodies may be more important in this role since the denture excludes, to a varying extent, salivary antibodies. Antigen-antibody reactions are probably responsible for the inflammation seen. When anti-fungal treatment is instituted the number of yeast organisms is reduced, cellular immunity is restored and together with humoral immunity the two may contribute to clearing of the infection. However following cessation of antifungal treatment, if re-infection is rapid, antibody levels will be sustained and cellular immunity will again decline. In tobacco smokers cellular immunity may be further suppressed and in addition the humoral immune response is also suppressed. These patients therefore respond more slowly to antifungal therapy and denture stomatitis may only

show signs of resolution once yeast numbers have been considerably reduced by the drug.

If antigen-antibody reactions are responsible for inflammation in denture stomatitis then initial inflammation ought to have been less in smokers than in non-smokers. It could be argued that this was indeed the case. Although the hyperplastic/granular form of the condition was far commoner amongst smokers than non-smokers this type of denture stomatitis is not necessarily associated with the greatest degree of erythema. The diffuse erythematous form of the disease was twice as common in non-smokers as smokers. Smoking may therefore be associated with apparently less severe inflammation but the inflammation which is seen in smokers may nonetheless be more resistant to treatment.

It is interesting to note that in a previous report, suppression of cellular immunity was most marked in patients with the hyperplastic/granular form of denture stomatitis[178] and in the present study hyperplastic/granular disease was found most commonly amongst smokers. In the previous study the influence of smoking was not considered. It is possible that smoking is independently associated with both hyperplastic/granular denture stomatitis and with impaired immune responses. However it is also possible

that the association between smoking and the hyperplastic/granular form of the disease is mediated by the immune response. A recent report has indicated that dentate patients with AIDS may develop paplillary hyperplasia of the palate associated with candidal infection[254]. The patients were all severely immunocompromised and had long standing oral candidosis. It seems possible therefore that mucosal proliferation may occur in patients with impaired immunity in response to chronic yeast infection, possibly as a defence mechanism.

Much of the work which has been done on denture stomatitis has concentrated on the external causes of the disease, such as yeast infection and denture trauma. Yeasts, present largely in denture plaque, would seem to be the most important of these external causes. However it is clear that the balance between host and parasite is of fundamental importance in this disease. Any factor which affects the host defence mechanisms may influence the onset of the disease and its response to treatment. Unfortunately the host response is frequently beyond the clinicians control but controlling the parasite is more feasible. Treatment of denture stomatitis must aim to reduce levels of denture plaque. Antifungal drugs may be a useful adjunct but, in the absence of good denture hygiene, re-infection and clinical failure are almost inevitable.

CONCLUSIONS

The results of the investigations described in this thesis suggest the following conclusions:

1. To obtain long term clinical success in the treatment of denture stomatitis, antifungal agents must achieve elimination or near elimination of yeast organisms from the mouth and this mycologic status must be sustained.

2. Antifungal agents rarely achieve complete elimination of yeasts from denture and mucosal surfaces.

3. Where any yeasts remain after antifungal therapy, effective denture hygiene measures are probably crucial in preventing repopulation of denture and mucosal surfaces.

4. The necessary degree of reduction in yeast numbers can probably be more reliably demonstrated by absence of growth from swabs of the palatal mucosa and denture fitting surface than from reduction in colony counts derived from oral rinse samples, whether or not the denture is worn during rinsing.

5. Fluconazole and amphotericin are equally efficacious in the treatment of denture stomatitis. Neither drug is associated with any serious or frequent side effect.

6. The response of denture stomatitis to antifungal treatment is poorer in smokers than in non-smokers.

7. The humoral immune response is depressed in smokers compared with non-smokers. This may explain the poorer clinical response of smokers to antifungal therapy.

8. The hyperplastic/granular form of denture stomatitis is commoner in smokers than in non-smokers. This may be related to the depressed immunity seen in smokers.

9. The quality of the dentures themselves does not appear to influence the outcome of antifungal treatment of denture stomatitis. By implication, therefore, denture trauma is probably of only secondary importance in the aetiology of the condition.

10. Chronic candidal infection may cause suppression of the cellular immune response but this appears to be limited specifically to the ability to respond to *Candida* antigen.

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APPENDIX 1.

Enzyme-Linked Immunosorbent Assay: Reagents.

The preparation of the reagents was essentially the same as that described by Wray[235]. The coating buffer for sensitising the the plates, a carbonate-bicarbonate buffer (pH 9.6), consisted of 1.59 gms of Na_2CO_3, 2.93 gms of $NaHCO_3$ and 0.2 gms of NaN_3 made up to one litre with distilled water. This was stored at 4 °C for not more than two weeks. The phosphate buffered saline-tween 20 mixture (PBS-tween) consisted of 8 gms of NaCl, 0.2 gms of KH_2PO_4, 2.9 gms $Na_2HPO_4.12H_2O$, 0.2 gms of KCl and 0.5 mls of tween-20 (Sigma, Poole, Dorset, UK) in one litre of distilled water. The pH of the solution was 7.4. For alkaline phosphatase conjugates, p-nitrophenyl phosphate tablets (Sigma 104 phosphate substrate tablets, 5mg, Sigma, Poole, Dorset, UK) were used for convenience. Immediately before use, one 5 mg tablet was dissolved in each 5 mls of diethanolamine buffer already warmed to room temperature. The diethanolamine buffer contained 97 mls of diethanolamine (Sigma, Poole, Dorset, UK) , 800 mls of distilled water, 0.2 gms of NaN_3 and 100 mg of $MgCl_2.6H_2O$; 1 M HCl was added until the pH reached 9.8, and then distilled water was added to a final volume of one litre.

APPENDIX 2.

Complete crosstabulations of symptom severity at each follow-up visit, compared with baseline severity.

Clinical symptoms : summary table

Visit=Visit 2 (7 days)

Clinical symptom Pain

	Fluconazole	Amphotericin	
	Severity of symptom	Severity of symptom	
	Absent	Absent	Mild
Baseline severity			
Absent	22	24	0
Mild	2	0	0
Moderate	1	0	1
Severe	1	0	0

Clinical symptoms : summary table

Visit=Visit 3 (1 month)

Clinical symptom Pain

	Fluconazole		Amphotericin
	Severity of symptom		Severity of symptom
	Absent	Mild	Absent
Baseline severity			
Absent	21	0	20
Mild	1	1	0
Moderate	1	0	1
Severe	1	0	0

Clinical symptoms : summary table

Visit=Visit 4 (3 months)

Clinical symptom Pain

	Fluconazole		Amphotericin	
	Severity of symptom		Severity of symptom	
	Absent	Mild	Absent	Moderate
Baseline severity				
Absent	23	0	21	0
Mild	0	2	0	0
Moderate	1	0	1	1

Clinical symptoms : summary table

Visit=Visit 2 (7 days)

Clinical symptom Burning

	Fluconazole		Amphotericin		
	Severity of symptom		Severity of symptom		
	Absent	Mild	Absent	Mild	Moderate
Baseline severity					
Absent	19	0	18	1	0
Mild	3	1	1	1	0
Moderate	1	0	0	0	3
Severe	2	0	0	1	0

Clinical symptoms : summary table

Visit=Visit 3 (1 month)

Clinical symptom Burning

	Fluconazole	Amphotericin		
	Severity of symptom	Severity of symptom		
	Absent	Absent	Mild	Moderate
Baseline severity				
Absent	18	14	0	0
Mild	4	1	1	0
Moderate	1	0	1	3
Severe	2	0	1	0

Clinical symptoms : summary table

Visit=Visit 4 (3 months)

Clinical symptom Burning

	Fluconazole		Amphotericin		
	Severity of symptom		Severity of symptom		
	Absent	Moderate	Absent	Mild	Moderate
Baseline severity					
Absent	20	0	16	1	0
Mild	4	0	2	0	0
Moderate	0	1	0	1	2
Severe	0	1	0	0	1

Clinical symptoms : summary table

Visit=Visit 2 (7 days)

Clinical symptom Abnormal taste

	Fluconazole			Amphotericin		
	Severity of symptom			Severity of symptom		
	Absent	Mild	Severe	Absent	Mild	Severe
Baseline severity						
Absent	18	0	1	21	1	0
Mild	3	1	0	1	1	0
Moderate	2	0	0	0	0	0
Severe	0	0	1	0	0	1

Clinical symptoms : summary table

Visit=Visit 3 (1 month)

Clinical symptom Abnormal taste

	Fluconazole				Amphotericin	
	Severity of symptom				Severity of symptom	
	Absent	Mild	Moderate	Severe	Absent	Severe
Baseline severity						
Absent	17	1	0	0	18	0
Mild	2	0	1	0	2	0
Moderate	2	1	0	0	0	0
Severe	0	0	0	1	0	1

Clinical symptoms : summary table

Visit=Visit 4 (3 months)

Clinical symptom Abnormal taste

	Fluconazole			Amphotericin		
	Severity of symptom			Severity of symptom		
	Absent	Mild	Moderate	Absent	Mild	Severe
Baseline severity						
Absent	20	0	0	20	0	0
Mild	2	0	1	1	1	0
Moderate	1	1	1	0	0	0
Severe	0	0	0	0	0	1

Clinical symptoms : summary table

Visit=Visit 2 (7 days)

Clinical symptom Xerostomia

	Fluconazole			Amphotericin			
	Severity of symptom			Severity of symptom			
	Absent	Mild	Severe	Absent	Mild	Moderate	Severe
Baseline severity							
Absent	18	0	0	14	2	0	0
Mild	3	2	1	3	3	0	0
Moderate	1	0	0	0	1	1	0
Severe	1	0	0	0	0	0	1

Clinical symptoms : summary table

Visit=Visit 3 (1 month)

Clinical symptom Xerostomia

	Fluconazole			Amphotericin			
	Severity of symptom			Severity of symptom			
	Absent	Mild	Moderate	Absent	Mild	Moderate	Severe
Baseline severity							
Absent	17	0	0	13	0	0	0
Mild	5	1	0	3	2	0	0
Moderate	1	0	0	0	1	1	0
Severe	0	0	1	0	0	0	1

Clinical symptoms : summary table

Visit=Visit 4 (3 months)

Clinical symptom Xerostomia

	Fluconazole		Amphotericin			
	Severity of symptom		Severity of symptom			
	Absent	Mild	Absent	Mild	Moderate	Severe
Baseline severity						
Absent	19	0	13	0	0	0
Mild	5	1	5	2	0	0
Moderate	1	0	0	1	1	0
Severe	0	0	0	0	0	1

APPENDIX 3.

Analytical Profile Index System.

Selected colonies were sampled using a sterile wooden applicator which was then used to inoculate molten Sabouraud's medium. This suspension was then inoculated into each of the plastic cupules on the API strip. One cupule contained this suspension only, and served as a control, the remainder contained dehydrated carbohydrate substrates. The strips were incubated at 30 °C for 72 hours. The ability of the yeast sample to assimilate each substrate was indicated by an increase in turbidity greater than that demonstrated by the control. The assimilation reactions of each test sample were used to produce a profile number, according to the manufacturer's instructions, which was then compared with the profile index provided, in order to identify the organism.

APPENDIX 4.

Publications arising.

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ORAL MEDICINE

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Comparative trial of fluconazole and amphotericin in the treatment of denture stomatitis

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The efficacy of fluconazole and amphotericin in the management of denture stomatitis was investigated in a comparative trial. Patients were assessed clinically, hematologically, and mycologically at the time of entry into the study and at 1, 4, and 12 weeks thereafter. A total of 29 patients were selected at random to receive 50 mg of fluconazole daily for 14 days; 30 patients were selected to receive amphotericin lozenges and cream for 28 days. Clinical response rates were similar in both treatment groups throughout the study. The best mycologic response was noted after 1 week whereas the best clinical response was observed after 4 weeks. Clinical evidence of relapse and recurrence at 12 weeks was a common finding irrespective of treatment. Side effects were uncommon in both groups. (ORAL SURG ORAL MED ORAL PATHOL 1993;76:35-9)

Denture stomatitis is a condition characterized by erythema of the denture-bearing mucosa, which occurs almost exclusively in the maxilla. Newton[1] described three types on the basis of clinical appearance, and this classification was later modified by Budtz-Jorgensen and Bertram[2] who chose the terms simple localized, simple diffuse, and granular to describe the inflammatory changes seen. The prevalence of the condition among denture wearers has been variously reported as between 25% and 65% depending on the population studied.[3-6] The condition may therefore be considered common.

The cause of denture stomatitis is often said to be multifactorial, but denture trauma, denture plaque, and candidal infection seem to be among the most important causes.[7] Consequently treatment includes correcting denture faults, improving denture hygiene, and, although some would question its necessity, antifungal therapy.

Up to this time, the antifungal agents used have been the polyenes—nystatin and amphotericin, and the imidazole—miconazole, all topically applied.[8] Reports suggest that these drugs are effective in producing an initial improvement in the condition but that recurrence rates are high and treatment regimens tend to be prolonged.[9-14] Systemically administered imidazoles, such as ketoconazole, have not been used routinely because of the superficial nature of the lesion and the possibility of hepatotoxic reactions.

Fluconazole is a recently introduced, bis-triazole, systemic antifungal agent, which is very well absorbed orally. It is widely distributed throughout body fluids including saliva and has an elimination half-life of approximately 30 hours.[15] Fluconazole has been shown to be effective in the management of superficial fungal infections such as vaginal candidiasis[16] and oropharyngeal candidiasis in patients who are HIV-positive.[17, 18] A feature of these reports has been the rapidity of clinical improvement observed. Lamey et al.[19] described the successful use of fluconazole to treat a single patient with chronic hyperplastic candidiasis. Fluconazole has been shown to be efficacious in the treatment of denture stomatitis in one open, noncomparative study[20] and in one placebo-con-

Trial sponsored by Pfizer. D.H.F. supported by the Medical Research Council (G84/2187).
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trolled trial.[21] Thus far there has been no comparative trial of fluconazole and a standard therapeutic regimen in the treatment of denture stomatitis.

The aims of this study were to determine the efficacy of systemic fluconazole versus topical amphotericin in the treatment of denture stomatitis and the relative recurrence rates after each treatment.

METHODS

Diagnosis

Patients were entered into the trial on the basis of a clinical diagnosis of denture stomatitis and mycologic investigation for the presence of yeast organisms. The clinical appearance was classified as localized erythematous, diffuse erythematous, or hyperplastic granular. (Localized erythematous was defined as a patchy form of erythema rather than the pin-point hyperemia described by Newton as type I denture stomatitis.)

Patients

Fifty-nine patients with denture stomatitis were recruited after explanation and consent from those who attended the Prosthetics Department of Edinburgh Dental Hospital. Of these 16 were men with a mean age of 51 years (range, 22 to 77 years) and 43 were women with a mean age of 55 years (range, 26 to 81 years). Patients were excluded from the study for the following reasons:

1. If they were pregnant, lactating, or of child-bearing age and not using reliable contraception
2. If they had impaired renal or hepatic function
3. If they had used any other antifungal agent during the past 10 days
4. If they were taking barbiturates, coumarin anticoagulants, or oral hypoglycemic agents
5. If they had a known sensitivity to polyenes or the azole group of antimycotics
6. If they intended to donate blood during or 3 weeks after the study period
7. If they had a history of alcoholism, drug abuse, psychiatric disorder, or any other problem that might invalidate informed consent

Trial design

At the initial visit patients were assessed clinically and mycologically, and blood was obtained for hematologic investigations (vide infra). They were then randomly assigned to receive either 50 mg of fluconazole daily for 14 days or 40 mg lozenges of amphotericin four times a day plus amphotericin cream to place on the fitting surface of the upper denture for 28 days. All patients received instruction in denture hygiene.

Patients were reviewed at 1 week, 4 weeks, and 12 weeks after treatment commenced. The same clinician examined all patients at all visits and was unaware of the medication prescribed.

Clinical assessment

Denture stomatitis was classified as aforementioned. Clinical symptoms of burning, xerostomia, pain, and abnormal taste were recorded as absent, mild, moderate, or severe at each visit.

At review visits the palatal tissues were examined, and denture stomatitis, if present, was classified. A subjective assessment was made of the extent of any reduction in erythema. A note was made when it was thought a distinct clinical improvement had taken place. Adverse events were noted.

Mycologic assessment

At each visit swabs were taken from the palate, fitting surface of the denture, tongue, floor of mouth, and the right and left angles of the mouth. A phosphate-buffered saline rinse sample was obtained for quantitative analysis of yeast organisms.[22] These samples were transported immediately to the laboratory where they were cultured with the use of standard techniques.

Hematologic and biochemical investigations

A full blood cell count and estimates of serum ferritin, serum folate, and serum B_{12} were obtained. Liver function tests were also performed. The blood count and liver function tests were repeated at follow-up visits.

RESULTS

Of the 59 patients who entered the study, 29 were randomly selected to receive fluconazole and 30 to receive amphotericin. Fifteen patients in the fluconazole group were smokers as were 10 in the amphotericin group. The demography of each treatment group and the numbers with each type of denture stomatitis at the initial assessment are summarized in Tables I and II.

All patients except one had yeast organisms recovered from their mouths, with *Candida albicans* by far the most common yeast species isolated. The patient from whom no yeast organisms were isolated responded to treatment and was therefore included in the clinical efficacy analysis although not in the mycologic analysis.

Nine patients failed to complete the study according to the protocol, but only three patients were excluded from all efficacy analyses: two amphotericin patients for poor compliance and one fluconazole patient who failed to return after the baseline visit.

Clinical findings

The number of patients cured or improved in each treatment group is given in Table III. The differences between treatments were not significant at any visit. The best cure rate was seen at 4 weeks with approximately 44% of patients in the fluconazole group cured and 63% in the amphotericin group clinically cured. If those cured and those who showed a marked clinical improvement are considered together, greater efficacy is observed: after 1 week approximately 50% of patients were clinically cured or improved in both treatment groups. At 4 weeks this had risen to 84% for the fluconazole group and 90% for the amphotericin group. However, by 12 weeks, there had been considerable recurrence, and only about 50% of patients in each group remained cured or improved.

Very few patients experienced clinical symptoms, and these showed no change or were improved during the study. In this respect there was no significant difference between treatments at any visit.

Laboratory findings

Table IV details the mycologic response to treatment. Mycologic cure was taken to be complete eradication of all yeast organisms. An arbitrary level of 50% or greater reduction in colony-forming units per milliliter as derived from the PBS rinse was considered a mycologic improvement. The best mycologic response occurred at 1 week when 50% of the patients in the fluconazole group and 68% in the amphotericin group were cured or improved according to the criteria set. However, by 4 and 12 weeks, there had been progressive recolonization in both groups. For individual patients in both treatment groups, the correlation between mycologic events and clinical status was poor.

Hematologic investigations

At the outset five patients (four assigned to fluconazole and one to amphotericin) had a low serum ferritin and two patients (both receiving amphotericin) had a low serum folate. These deficiencies were not corrected, and despite this, only one patient, who had shown poor compliance, failed to respond to treatment. During treatment there were no adverse changes in hematologic indexes or liver function tests.

Side effects

The overall incidence of adverse effects during treatment was low. Only one patient, who was taking amphotericin, discontinued treatment as a result of nausea.

Table I. Demographic details for each treatment group

	Fluconazole	*Amphotericin*
Total number	29	30
Men	8	8
Women	21	22
Mean age (years)	50	60
Age range (years)	22-77	24-81

Table II. Classification of denture stomatitis in each treatment group at initial visit

	Fluconazole	*Amphotericin*
Localized erythematous	7	7
Diffuse erythematous	15	17
Hyperplastic granular	7	6
Total	29	30

DISCUSSION

The results of this study indicate that systemic fluconazole is as effective as topical amphotericin in the treatment of denture stomatitis and confirm that fluconazole is a safe, well-tolerated drug. The figures of 84% of patients cured or improved by fluconazole correspond well with the 16% cured and 73% improved reported by Budtz-Jorgensen et al.[21] The higher proportion of patients cured in the present study (44%) may be explained by the fact that the patients were advised on denture hygiene and told to leave their dentures out at night, whereas in the aforementioned study the patients were given no such instructions.

In the present study the clinical relapse and recurrence rates were similar in both treatment groups with only about half of the patients remaining cured or improved at the 12-week follow-up visit. This is consistent with other reports that demonstrate high rates of relapse and recurrence of denture stomatitis after antifungal therapy,[10, 14, 23-25] and there are a number of possible explanations for this.

The relationship between denture plaque, yeasts, and denture stomatitis seems to be established.[14, 23, 26, 27] The denture must therefore constitute a potential source of reinfection, and this view has been expressed by other authors.[10, 24] It follows that successful long-term treatment of denture stomatitis depends upon either the complete removal of denture plaque, which is preferable, or the eradication of yeasts within the plaque. Both objectives require patient compliance, in the first case with oral hygiene measures and in the second case with the treatment regimen. In addition the therapeutic agent must reach

Table III. Clinical response

	1 Week		*4 Weeks*		*12 Weeks*	
	F	*A*	*F*	*A*	*F*	*A*
Cured	5	9	11	14	6	5
Improved*	8	6	10	6	9	7
No change*	13	11	4	2	11	12
Total	26	26	25	22	26	24

F, fluconazole
A, amphotericin
*Improved and no change are in comparison with the baseline assessment.

Table IV. Mycologic response

	1 Week		*4 Weeks*		*12 Weeks*	
	F	*A*	*F*	*A*	*F*	*A*
Cured	8	12	3	10	4	1
Improved*	5	5	2	2	0	8
No change*	13	8	20	9	22	14
Total	26	25	25	21	26	23

F, fluconazole
A, amphotericin
*Improved and no change are in comparison with the baseline assessment.

the site of infection and yeast proliferation, namely the palatal mucosa and the fitting surface of the denture.

Only two patients showed poor compliance with treatment, and both were in the amphotericin group. Subjectively it seems reasonable to expect patients to comply more readily with taking one capsule daily than with sucking four lozenges and spreading cream on the fitting surface of the denture.

The therapeutic effect of fluconazole on superficial oral mucosal lesions is probably dependent on its presence in saliva. If saliva is unable to flow freely below the fitting surface of a tightly fitting denture, this might restrict the effectiveness of fluconazole. For this reason, removing the dentures at night must be considered an important adjunct to fluconazole treatment. The patients treated with amphotericin, as well as sucking lozenges also placed amphotericin cream on the fitting surface of the denture. Amphotericin may therefore have had better access to the denture plaque than fluconazole. In either treatment group, failure to remove yeasts completely from the denture-fitting surface or the lesion would predispose the patient to recurrence of the condition after cessation of treatment.

Reinfection from exogenous sources[21] and from the gut[25] has also been suggested as a cause of relapse. Systematically administered fluconazole might be expected to more completely clear *Candida* from other host sites.

It is difficult to draw conclusions about the cause of relapse and recurrence after antifungal therapy except to say that it is probably a result of a combination of the factors discussed.

Many studies of antifungal therapy in the treatment of denture stomatitis have involved an assessment of the effect on mycologic parameters.[10-13, 21, 23-25, 27, 28] In the present study the best mycologic cure rates were seen 1 week into treatment, but at subsequent follow-up visits there was progressive recolonization. These findings are in agreement with previous reports.[23, 25, 28] Previous attempts to assess mycologic improvement in patients not rendered yeast-free have been based on determining the reduction in the density of growth.[11-13, 21, 23, 25, 27, 28] However, many of the methods used have been only semiquantitative.[13, 21, 25] Methods of quantitative assessment have been compared, and the imprint culture and concentrated rinse culture methods were found to be equally sensitive.[22]

In this study with the use of a concentrated oral rinse culture and the criteria described, mycologic improvement was not suprisingly more prolonged in the amphotericin group that received a longer course of treatment. In light of this it might be suggested that fluconazole treatment should be continued longer. However, it could also be argued that there is little value in viewing the mycologic results in isolation, especially because the present study and others[21, 28] have found poor correlation between mycologic events

and the observed clinical status. This has been attributed to the involvement of factors other than *Candida* in the cause of denture stomatitis,[28, 29] but it might equally be due to differing host responses.

Given the high rates of relapse and recurrence together with the progressive recolonization by yeasts, should antifungals be used to treat denture stomatitis? The condition is often diagnosed when patients request new dentures, and because inflamed tissues offer poor support for prostheses, treatment is justified. However, treatment should be directed primarily toward reducing levels of denture plaque. In patients who do not respond to this approach or in patients who are at risk of disseminated infection, antifungal treatment may be required. In these circumstances fluconazole is a convenient and safe therapeutic agent. The efficacy of fluconazole and the improved patient compliance associated with its use also has implications with respect to the treatment of other oral candidiasis.

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Humoral immune responses in *Candida*-associated denture stomatitis.

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Previous studies have demonstrated an increased incidence of *Candida* carriage and infection among cigarette smokers when compared to non-smokers. However the mechanisms underlying this difference remain unclear. The aim of the present study was to investigate the effects of cigarette smoking on the immune response in patients with *Candida*-associated denture stomatitis. Fifty two patients (24 smokers, 28 non-smokers) were investigated. Samples of serum and whole saliva were obtained before the start of treatment and at one, four and twelve weeks thereafter. Total serum and salivary IgG and IgA concentrations were measured by a commercially available radial immunodiffusion assay (Behringwerke AG, Marburg, Germany); serum and salivary anti-*Candida* IgG and IgA levels were measured using a double sandwich ELISA technique. Data was analysed using Mann-Whitney and Wilcoxon rank sum non-parametric tests. Before the start of treatment serum anti-*Candida* IgG levels in non-smokers (476 $\pm$ 98[S.E.]) were significantly higher than in smokers (170 $\pm$ 30); $p=0.001$. A similar difference was observed at all other times throughout the study. Total serum IgG levels were higher among non-smokers at baseline and week one ($p<0.05$). Total serum IgA concentrations were higher among non-smokers at weeks one, four and 12 ($p<0.05$). No significant differences were observed in salivary immunoglobulin concentrations. This study provides evidence of the effects of cigarette smoking on the immune response and may help to explain the higher incidence of *Candida* carriage and infection seen in smokers.